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SERIES B

CONTAINING PAPERS OF A BIOLOGICAL CHARACTER

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THE ROYAL SOCIETY.

Section B.—Biological Sciences.

Trypanosome Diseases of Domestic Animals in Nyasaland.

III.—Trypanosoma pecorum.

By Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S.; Majors David Harvey and A. E. Hamerton, D.S.O., R.A.M.C.; and Lady Bruce, R.R.C.

(Scientific Commission of the Royal Society, Nyasaland, 1913.)

(Received April 14,—Read May 8, 1913.)

Introduction.

This trypanosome has been found in the neighbourhood of the camp at Kasu, in cattle, wild game and wild tsetse flies. In a herd of cattle belonging to the Mvera Mission, which lies about two miles to the east, 32 per cent. were found to be infected by this disease. The mission station is built near the edge of the "fly-country," and there is little doubt that the cattle were at times exposed to the bite of the "fly." After the disease had been discovered to be present in the herd the animals were prevented from grazing in the direction of the danger, and since then no new cases have occurred. It is also the species of trypanosome most commonly found in the blood of the wild game in this district, and consequently the tsetse fly is found infected with it more frequently than with any other. It is one of the most important trypanosome diseases of domestic animals in Central Africa, as it affects them all—horses, cattle, goats, sheep, pigs, and dogs.

Morphology of Trypanosoma pecorum.

The description already given* of this species of trypanosome as regards its movements and appearance when alive, its shape, contents of cell, etc., when stained, are applicable to the species as it occurs in Nyasaland and need not be repeated.

---

* 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 468.

I. *The Mvera Cattle Strain.*

Length.—The following table gives the length of this trypanosome as found in the donkey, ox, goat, dog, and rat—500 trypanosomes in all:—

Table I.—Measurements of the Length of the Trypanosome of the Mvera Cattle Strain.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912.							
Jan. 25	70	Donkey	Osmic acid	Giemsa	14·1	16·0	11·0
March 15	291	,,	,,	,,	12·9	16·0	11·0
April 16	445	,,	,,	,,	14·0	16·0	12·0
,, 18	291	,,	,,	,,	13·0	15·0	12·0
Jan. 17	102	Ox	,,	,,	14·8	17·0	13·0
,, 17	103	,,	,,	,,	13·9	16·0	12·0
Feb. 9	100	,,	,,	,,	13·0	15·0	11·0
,, 9	103	,,	,,	,,	14·6	17·0	11·0
,, 9	103	,,	,,	,,	14·5	17·0	12·0
,, 20	104	,,	,,	,,	14·0	16·0	12·0
,, 26	100	,,	,,	,,	14·4	16·0	11·0
March 1	227	,,	,,	,,	14·0	16·0	12·0
,, 1	228	,,	,,	,,	14·5	18·0	11·0
Feb. 26	208	Goat	,,	,,	13·2	15·0	9·0
,, 29	178	,,	,,	,,	13·2	15·0	10·0
March 11	206	,,	,,	,,	14·6	17·0	11·0
,, 11	208	,,	,,	,,	14·6	16·0	12·0
May 20	199	,,	,,	,,	13·3	15·0	12·0
Feb. 15	16	Dog	,,	,,	13·8	16·0	11·0
,, 26	212	,,	,,	,,	14·4	16·0	13·0
,, 26	214	,,	,,	,,	13·3	16·0	11·0
,, 26	217	,,	,,	,,	13·5	15·0	12·0
March 4	218	,,	,,	,,	13·8	16·0	12·0
,, 14	223	Rat	,,	,,	14·8	16·0	13·0
,, 18	223	,,	,,	,,	14·7	16·0	13·0
					14·0	18·0	9·0

The average length of the trypanosome of the Mvera Cattle strain, in different species of animals, is as follows (Table II):—

Rats were, as a rule, immune to this strain, but on one occasion, in a case of mixed infection, it was found along with *T. brucei*. It was thought that the presence of *T. brucei* may have broken down the rat's immunity to *T. pecorum*.

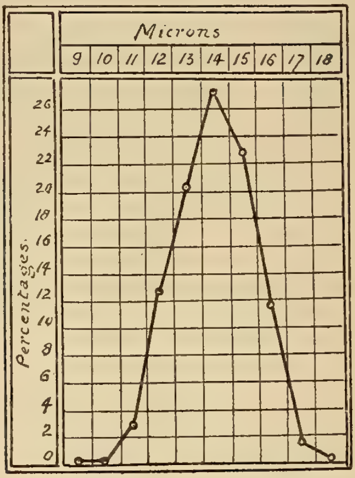
Table II.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Donkey	80	13·5	16·0	11·0
Ox	180	14·2	17·0	11·0
Goat..................	100	13·8	17·0	9·0
Dog	100	13·8	16·0	11·0
Rat	40	14·8	16·0	13·0

Table III.—Distribution in respect to Length of 500 Individuals of the Mvera Cattle Strain.

Animal.	In microns.											Average length.
	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	
Donkey	—	—	1	2	5	2	6	4	—	—	—	14·1
,, ...	—	—	1	8	5	4	1	1	—	—	—	12·9
,, ...	—	—	—	2	4	8	4	2	—	—	—	14·0
,, ...	—	—	—	9	3	6	2	—	—	—	—	13·0
Ox	—	—	—	—	6	4	7	2	1	—	—	14·8
,, ...	—	—	—	4	5	3	4	4	—	—	—	13·9
,, ...	—	—	3	4	6	3	4	—	—	—	—	13·0
,, ...	—	—	2	1	1	6	5	2	3	—	—	14·6
,, ...	—	—	—	1	2	8	5	3	1	—	—	14·5
,, ...	—	—	—	2	5	5	7	1	—	—	—	14·0
,, ...	—	—	1	2	1	5	5	6	—	—	—	14·4
,, ...	—	—	—	3	4	5	6	2	—	—	—	14·0
,, ...	—	—	1	1	3	5	6	1	2	1	—	14·5
Goat	1	—	—	4	5	7	3	—	—	—	—	13·2
,, ...	—	1	2	1	8	4	4	—	—	—	—	13·2
,, ...	—	—	—	1	4	4	4	7	—	—	—	14·6
,, ...	—	—	1	2	1	4	5	6	1	—	—	14·6
,, ...	—	—	—	6	4	7	3	—	—	—	—	13·3
Dog	—	—	1	4	2	6	5	2	—	—	—	13·8
,, ...	—	—	—	—	4	6	7	3	—	—	—	14·4
,, ...	—	—	2	3	5	7	2	1	—	—	—	13·3
,, ...	—	—	—	2	9	6	3	—	—	—	—	13·5
,, ...	—	—	—	2	7	5	4	2	—	—	—	13·8
Rat	—	—	—	—	1	7	7	5	—	—	—	14·8
,, ...	—	—	—	—	1	9	5	5	—	—	—	14·7
Totals	1	1	15	64	101	136	114	59	8	1	—	
Percentages	0·2	0·2	3·0	12·8	20·2	27·2	22·8	11·8	1·6	0·2	—	

Chart 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *Trypanosoma pecorum*—The Mvera Cattle Strain.



This curve is made up of measurements from 80 specimens of trypanosomes taken from the donkey, 180 from the ox, 100 from the goat, 100 from the dog, and 40 from the rat.

Breadth.—Measured across the broadest part of the body, but not including the undulating membrane, the Mvera Cattle strain of *T. pecorum* averages 1·97 microns (maximum 3, minimum 1·25).

II. *The Wild-game Strain.*

The citrated blood of the wild game was brought up from the "fly-country," by a motor-cyclist, and at once injected into a goat, monkey and dog. A description of the kind of wild game found in this locality and the trypanosomes they are infected with was given in a previous paper.*

Length.—The following table gives the length of this trypanosome as found in the goat, monkey, dog, and rat, 500 trypanosomes in all:—

---

* 'Roy. Soc. Proc.,' 1913, B, vol. 86, p. 269.

Table IV.—Measurements of the Length of *Trypanosoma pecorum*—The Wild-game Strain.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912.							
Feb. 1...	45	Goat	Osmic acid	Giemsa	15·7	17·0	13·0
Aug. 26...	898	,, ...	,,	,,	14·6	17·0	12·0
Sept. 2...	1031	,, ...	,,	,,	14·1	16·0	12·0
,, 9...	1037	,, ...	,,	,,	13·9	16·0	12·0
,, 9...	1039	,, ...	,,	,,	15·0	18·0	12·0
,, 9...	1121	,, ...	,,	,,	14·5	16·0	13·0
,, 12...	1039	,, ...	,,	,,	14·3	16·0	12·0
,, 23...	1383	,, ...	,,	,,	13·3	15·0	11·0
Oct. 7...	1307	,, ...	,,	,,	13·7	16·0	12·0
,, 7...	1383	,, ...	,,	,,	13·4	16·0	12·0
April 22...	403	Monkey	,,	,,	14·1	17·0	12·0
Sept. 9...	1085	,, ...	,,	,,	14·6	17·0	11·0
April 8...	355	Dog	,,	,,	14·2	17·0	12·0
,, 11...	356	,, ...	,,	,,	14·3	17·0	12·0
Aug. 19...	1015	,, ...	,,	,,	14·8	17·0	13·0
Sept. 2...	1086	,, ...	,,	,,	14·3	16·0	12·0
,, 9...	1086	,, ...	,,	,,	14·9	17·0	13·0
,, 12...	1015	,, ...	,,	,,	14·0	16·0	12·0
,, 12...	1086	,, ...	,,	,,	14·1	16·0	13·0
Nov. 7...	1510	,, ...	,,	,,	14·0	16·0	12·0
,, 18...	1510	,, ...	,,	,,	14·2	16·0	12·0
May 16...	511	Rat	,,	,,	14·3	18·0	12·0
Nov. 26...	1518	,, ...	,,	,,	14·1	16·0	12·0
Dec. 26...	1608	,, ...	,,	,,	15·0	18·0	13·0
,, 26...	1608	,, ...	,,	,,	14·6	17·0	13·0
					14·3	18·0	11·0

The average length of the trypanosome of the Wild-game strain, in different species of animals, is as follows:—

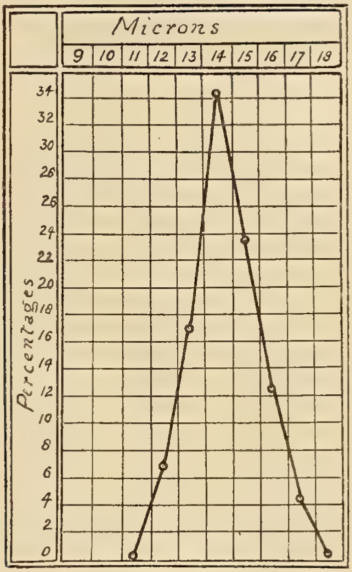
Table V.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat..................	200	14·2	18·0	11·0
Monkey	40	14·3	17·0	11·0
Dog	180	14·3	17·0	12·0
Rat	80	14·5	18·0	12·0

Table VI.—Distribution in respect to Length of 500 Individuals of the Wild-game Strain.

Animal.	In microns.								Average length.
	11.	12.	13.	14.	15.	16.	17.	18.	
Goat	—	—	1	4	6	5	4	—	15·7
”	—	1	2	7	6	2	2	—	14·6
”	—	1	3	11	3	2	—	—	14·1
”	—	1	8	5	3	3	—	—	13·9
”	—	1	2	3	7	4	2	1	15·0
”	—	—	4	7	4	5	—	—	14·5
”	—	2	—	10	5	3	—	—	14·3
”	1	4	4	10	1	—	—	—	13·3
”	—	1	8	8	2	1	—	—	13·7
”	—	5	5	8	1	1	—	—	13·4
Monkey	—	3	3	7	4	1	2	—	14·1
”	1	—	3	7	3	3	3	—	14·6
Dog	—	3	2	5	8	1	1	—	14·2
”	—	2	6	4	2	4	2	—	14·3
”	—	—	4	4	5	5	2	—	14·8
”	—	1	1	11	5	2	—	—	14·3
”	—	—	1	6	8	4	1	—	14·9
”	—	3	3	5	8	1	—	—	14·0
”	—	—	5	9	4	2	—	—	14·1
”	—	2	5	5	7	1	—	—	14·0
”	—	1	4	8	3	4	—	—	14·2
Rat	—	1	2	9	7	—	—	1	14·3
”	—	2	3	8	5	2	—	—	14·1
”	—	—	2	6	5	5	1	1	15·0
”	—	—	4	5	7	2	2	—	14·6
Total	2	34	85	172	119	63	22	3	
Percentages	0·4	6·8	17·0	34·4	23·8	12·6	4·4	0·6	

CHART 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. pecorum*, Wild-game Strain, taken at random from various animals.



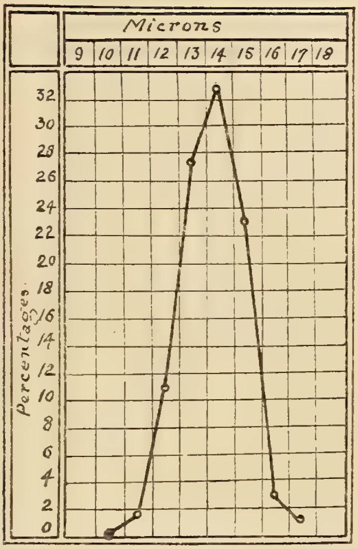
This curve is made up of measurements from 200 specimens of trypanosomes taken from the goat, 40 from the monkey, 180 from the dog, and 80 from the rat.

The following table gives the distribution in respect to length of *T. pecorum*, Wild-game strain, taken from a single rat on consecutive days, as was done in the description of the morphology of *T. brucei* vel *rhodesiense*. In this case the rat is not a suitable animal, since many strains of *T. pecorum* have no effect on it:—

Table VII.—Distribution in respect to Length of 500 Individuals of the Wild-game Strain, taken on nine consecutive days from Rat 510.

Animal.	In microns.								Average length.
	10.	11.	12.	13.	14.	15.	16.	17.	
Rat	—	—	2	2	7	6	2	1	14·3
,,	—	—	4	4	11	1	—	—	13·4
,,	—	1	1	8	8	2	—	—	13·4
,,	—	—	3	10	3	4	—	—	13·4
,,	—	1	1	9	6	3	—	—	13·4
,,	—	—	6	3	8	3	—	—	13·4
,,	—	—	1	8	6	4	1	—	13·8
,,	—	—	1	5	6	7	1	—	14·1
,,	—	—	2	7	4	7	—	—	13·8
,,	—	—	—	3	5	11	—	1	14·5
,,	—	2	1	4	9	4	—	–	13·6
,,	—	—	7	7	5	1	—	—	13·0
,,	—	—	5	4	9	1	1	—	13·4
,,	—	1	1	8	7	3	—	—	13·5
,,	1	—	3	8	3	5	—	—	13·3
,,	—	1	—	2	5	5	5	2	14·8
,,	—	—	—	10	5	5	—	—	13·7
,,	—	1	2	4	8	5	—	—	13·7
,,	—	—	1	3	8	7	—	1	14·2
,,	—	—	1	6	9	4	—	—	13·8
,,	—	—	—	2	7	8	3	—	14·6
,,	—	2	1	5	4	8	—	—	13·7
,,	—	—	5	4	9	2	—	—	13·4
,,	—	—	3	6	5	6	—	—	13·7
,,	—	—	2	5	6	5	1	1	14·0
Total	1	9	53	137	163	117	14	6	
Percentages	0·2	1·8	10·6	27·4	32·6	23·4	2·8	1·2	

Chart 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. pecorum*, Wild-game Strain, taken on nine consecutive days from Rat 510.



Breadth.—*T. pecorum*, Wild-game strain, measured across the broadest part of the body, not including the undulating membrane, averages 1·97 micron (maximum 2·75, minimum 1·25).

III. *The Wild* Glossina morsitans *Strain.*

The tsetse flies were brought from the neighbouring "fly-country" and fed on goats, monkeys, and dogs as described in a former paper.*

Length.—The following table gives the length of this trypanosome as found in the goat, monkey, dog, guinea-pig, and rat, 500 trypanosomes in all:—

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* 'Roy. Soc. Proc.,' 1913, B, vol. 86, p. 408.

Table VIII.—Measurements of the Length of *T. pecorum*—The Wild *Glossina morsitans* Strain.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912.							
Feb. 15...	89	Goat	Osmic acid	Giemsa	12·5	14·0	10·0
,, 19...	89	,, ...	,,	,,	13·9	17·0	11·0
Mar. 4...	89	,, ...	,,	,,	14·2	16·0	12·0
April 25...	363	,, ...	,,	,,	14·4	17·0	11·0
June 13...	422	,, ...	,,	,,	13·3	16·0	10·0
,, 17...	422	,, ...	,,	,,	13·6	16·0	11·0
,, 20...	564	,, ...	,,	,,	13·0	15·0	10·0
,, 24...	565	,, ...	,,	,,	13·3	15·0	11·0
July 1...	565	,, ...	,,	,,	12·9	15·0	9·0
,, 8...	634	,, ...	,,	,,	14·5	17·0	12·0
June 10...	450	Monkey	,,	,,	13·6	15·0	12·0
July 8...	623	,, ...	,,	,,	13·7	16·0	11·0
Mar. 9...	258	Dog	,,	,,	14·5	17·0	12·0
,, 18...	256	,, ...	,,	,,	13·5	16·0	11·0
,, 18...	258	,, ...	,,	,,	13·7	15·0	12·0
,, 18...	322	,, ...	,,	,,	13·4	15·0	11·0
,, 21...	256	,, ...	,,	,,	14·6	17·0	11·0
,, 21...	258	,, ...	,,	,,	14·1	17·0	12·0
,, 21...	322	,, ...	,,	,,	15·1	17·0	12·0
April 22...	400	,, ...	,,	,,	14·7	17·0	13·0
May 16...	452	Guinea-pig ...	,,	,,	14·6	18·0	12·0
,, 9...	454	Rat	,,	,,	13·9	17·0	12·0
,, 9...	455	,, ...	,,	,,	14·0	16·0	12·0
,, 16...	454	,, ...	,,	,,	13·7	16·0	12·0
,, 16...	455	,, ...	,,	,,	14·3	16·0	10·0
					13·9	18·0	9·0

The average length of the trypanosome of the Wild *Glossina morsitans* strain, in different species of animals, is as follows:—

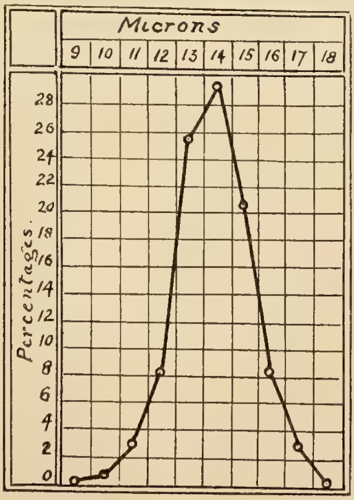
Table IX.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat..................	200	13·5	17·0	9·0
Monkey	40	13·6	16·0	11·0
Dog	160	14·2	17·0	11·0
Guinea-pig	20	14·6	18·0	12·0
Rat	80	14·0	17·0	10·0

Table X.—Distribution in respect to Length of the Wild *Glossina morsitans* Strain.

Animal.	In microns.										Average length.
	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	
Goat	—	1	3	5	6	5	—	—	—	—	12·5
,,	—	—	1	4	3	5	3	3	1	—	13·9
,,	—	—	—	2	1	9	6	2	—	—	14·2
,,	—	—	2	—	3	4	7	2	2	—	14·4
,,	—	1	3	1	3	9	2	1	—	—	13·3
,,	—	—	1	—	9	7	2	1	—	—	13·6
,,	—	1	—	4	8	6	1	—	—	—	13·0
,,	—	—	1	1	11	4	3	—	—	—	13·3
,,	1	—	1	3	8	6	1	—	—	—	12·9
,,	—	—	—	1	3	5	7	3	1	—	14·5
Monkey	—	—	—	2	6	10	2	—	—	—	13·6
,,	—	—	1	1	8	4	4	2	—	—	13·7
Dog	—	—	—	1	3	6	6	3	1	—	14·5
,,	—	—	1	2	8	5	2	2	—	—	13·5
,,	—	—	—	3	4	9	4	—	—	—	13·7
,	—	—	1	3	8	4	4	—	—	—	13·4
,,	—	—	1	2	2	4	3	5	3	—	14·6
,,	—	—	—	2	5	5	5	2	1	—	14·1
,,	—	—	—	1	—	3	10	4	2	—	15·1
,,	—	—	—	—	4	5	6	3	2	—	14·7
Guinea-pig	—	—	—	1	4	4	6	3	1	1	14·6
Rat	—	—	—	1	7	7	3	1	1	—	13·9
,,	—	—	—	1	5	7	6	1	—	—	14·0
,,	—	—	—	1	9	5	4	1	—	—	13·7
,,	—	1	—	—	1	9	6	3	—	—	14·3
Total	1	4	16	42	129	147	103	42	15	1	
Percentages ...	0·2	0·8	3·2	8·4	25·8	29·4	20·6	8·4	3·0	0·2	

Chart 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. pecorum*—The Wild *Glossina morsitans* Strain.



This curve is made up of measurements from 200 specimens of trypanosomes taken from the goat, 40 from the monkey, 160 from the dog, 20 from the guinea-pig, and 80 from the rat.

Breadth.—Measured across the broadest part of the body, not including the undulating membrane, *T. pecorum*, Wild *Glossina morsitans* strain, averages 1·94 micron (maximum 2·75, minimum 1·25).

COMPARISON OF THE MVERA CATTLE STRAIN, WILD-GAME STRAIN, AND WILD GLOSSINA MORSITANS STRAIN OF T. PECORUM.

The following table gives the average length of this trypanosome in the three strains under consideration, as found in the donkey, ox, goat, monkey, dog, guinea-pig, and rat:—

Table XI.—Measurements of the Length of the Trypanosomes of the Three Strains.

Date.	Strain.	No. of trypanosomes measured.	Animal.	In microns.		
				Average length.	Maximum length.	Minimum length.
1912	Mvera cattle	500	Various ...	14·0	18·0	9·0
1912	Wild game	500	,, ...	14·3	18·0	11·0
1912	Wild *G. morsitans* ...	500	,, ...	13·9	18·0	9·0

COMPARISON OF THE CURVES FROM THE THREE STRAINS.

CHART 5.—Curves representing the Distribution, by Percentages, in respect to Length, of *T. pecorum.* 500 specimens from each of the Mvera Cattle Strain, the Wild-game Strain, and the Wild *Glossina morsitans* Strain.

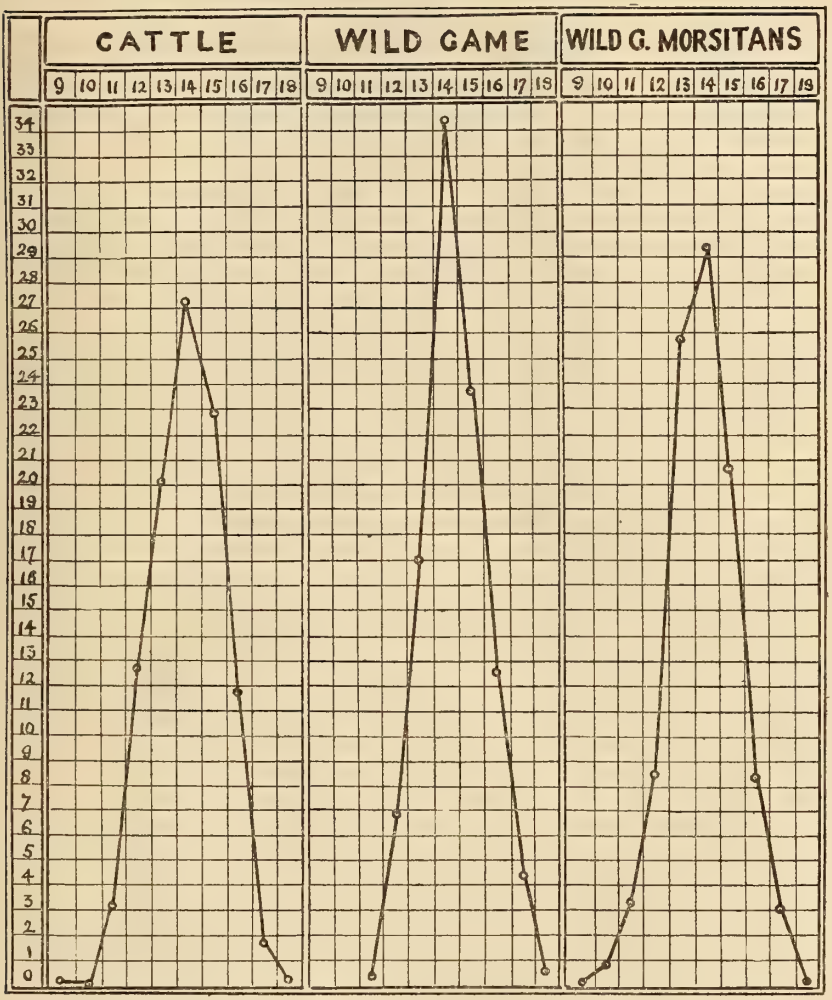


Table XII.—Distribution in respect to Length of 2000 Individuals of the Three Strains of *T. pecorum.* The trypanosomes have been taken at random from various animals.

	In microns.									
	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.
Totals	2	6	42	193	452	618	453	178	51	5
Percentages	0·1	0·3	2·1	9·6	22·6	30·9	22·7	8·9	2·5	0·3

Chart 6.—Curve representing the Distribution, by Percentages, in respect to Length, of 2000 Individuals of the Three Strains of *T. pecorum*, Nyasaland, described in this paper.

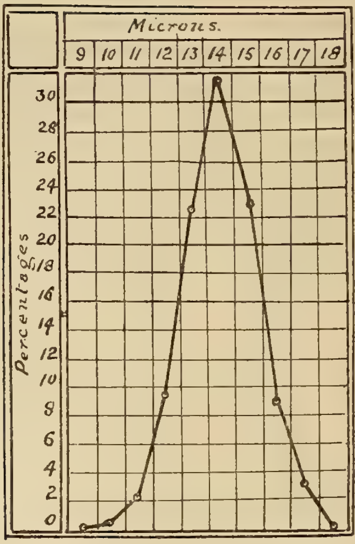


Table XIII.—Breadth of the Three Strains of *T. pecorum*. This does not include the undulating membrane.

Strain.	Average breadth.	Maximum breadth.	Minimum breadth.
Mvera cattle	1·97	3·0	1·25
Wild game..................	1·97	2·75	1·25
Wild *G. morsitans*	1·94	2·75	1·25
Average	1·96	2·83	1·25

ANIMALS SUSCEPTIBLE TO TRYPANOSOMA PECORUM.

I. *The Mvera Cattle Strain.*

Table XIV.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
		Donkeys.			
1912.					
Jan. 26...	291	Natural infection	?	—	Still alive after 377 days.
,, 26...	292	,, ...	?	87 ?	Died of *T. pecorum*, April 22.
June 18...	705	,, ...	?	—	Still alive after 233 days.
,, 18...	706	,, ...	?	—	,, 233 ,,
,, 18...	709	,, ...	?	—	,, 233 ,,
		Cattle.			
Jan. 16...	15 *a*	Natural infection	?	—	Still alive after 387 days.
,, 16...	15 *b*	,, ...	?	—	,, 387 ,,
,, 16...	15 *c*	,, ...	?	—	,, 387 ,,
,, 16...	15 *d*	,, ...	?	?	Died at Mvera of *T. pecorum*.
,, 16...	15 *e*	,, ...	?	?	,, ,,
,, 16...	15 *f*	,, ...	?	?	,, ,,
,, 16...	15 *g*	,, ...	?	?	,, ,,
,, 16...	15 *h*	,, ...	?	?	,, ,,
,, 16...	100	,, ...	?	78 ?	Died of *T. pecorum*, April 28.
,, 16...	101	,, ...	?	30 ?	,, ,, Feb. 15.
,, 16...	102	,, ...	?	—	Still alive after 387 days.
,, 16...	103	,, ...	?	201 ?	Died of *T. pecorum*, Aug. 28.
,, 16...	104	,, ...	?	—	Still alive after 387 days.
,, 16...	105	,, ...	?	—	,, 387 ,,
,, 16...	110	,, ...	?	?	Died at Mvera of *T. pecorum*.
,, 16...	174	,, ...	?	?	,, ,,
Feb. 16...	226	From Dog 16	11	—	Still alive after 356 days.
,, 16...	227	,, 16	7	—	,, 356 ,,
,, 16...	228	,, 16	11	—	,, 356 ,,
		Average......	10	103	
		Goats.			
Feb. 14...	203	From Cow 103	12	29	Died of *T. pecorum*.
,, 14...	205	,, 103	10	40	,, ,,
,, 14...	206	,, 103	15	35	,, ,,
,, 14...	208	,, 103	12	36	,, ,,
,, 16...	178	From Dog 16	12	38	,, ,,
,, 16...	199	,, 16	11	209	,, ,,
,, 16...	204	,, 16	10	—	Still alive after 356 days.
		Average......	12	64	
		Dogs.			
Jan. 17...	16	From Cow 102	26	30	Died of *T. pecorum*.
,, 17...	17	,, 103	13	56	,, ,,
,, 17...	18	,, 101	—	—	Never showed trypanosomes.
,, 17...	19	,, 100	13	56	Died of *T. pecorum*.
Feb. 14...	217	,, 103	16	28	,, ,,
,, 16...	212	From Dog 16	6	26	,, ,,
,, 16...	214	,, 16	10	26	,, ,,
,, 16...	218	,, 16	17	22	,, ,,
,, 16...	225	,, 16	10	26	,, ,,
		Average......	14	34	

* Duration includes the days of incubation; it dates from day of infection.

Table XIV—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
		Guinea-pigs.			
1912. Feb. 14...	221	From Cow 103	—	—	Never showed trypanosomes.
„ 14...	222	„ 103	—	—	„ „
„ 16...	229	From Dog 16	—	—	„ „
„ 16...	230	„ 16	—	—	„ „
		Rats.			
Feb. 14...	224	From Cow 103	—	—	Never showed trypanosomes.
„ 16...	231	From Dog 16	—	—	„ „
„ 16...	232	„ 16	—	—	„ „
May 4...	508	From Cow 103	—	—	„ „
„ 4...	509	„ 103	—	—	„ „

* Duration includes the days of incubation; it dates from day of infection.

Disease set up in Various Animals by Trypanosoma pecorum, *Nyasaland—The Mvera Cattle Strain.*

Donkeys.—When the Commission arrived at Kasu in January, 1912, the neighbouring Mvera Mission Station owned a herd of 16 donkeys. On examining them five were found to be infected with *T. pecorum.* One died after 87 days; the other four are at present free from trypanosomes and apparently in good health. It is true that three of the apparently recovered animals were given arsenic for some months, but the fourth, which got none, did equally well. It may also be noted that two of the four have recently given birth to healthy foals. Susceptible animals inoculated with the blood of the four donkeys remained healthy. From this it would appear that this strain of *T. pecorum* is not very fatal to donkeys.

Cattle.—A year ago the Mvera herd consisted of 50 head of cattle, and of these 16 were found to be infected with *T. pecorum.* At the present date 10 of these have died of this disease and six remain alive. The six have shown trypanosomes in their blood for some months, and their blood inoculated into healthy dogs has proved non-infective. In spite of this, however, these cattle remain in poor condition: thin, with rough, staring coats, and every appearance of suffering from a chronic disease, and it seems highly probable that more of them, if not all, will succumb to the effects of the trypanosome. Three oxen (Experiments 226, 227, and 228), which were inoculated from a dog whose blood contained *T. pecorum,* at the present date appear quite healthy and have apparently recovered.

Goats.—The Mvera Cattle strain is fatal to goats, killing them, as a rule, in 30 to 40 days. One goat lived 209 days, and another is still alive after a year and appears to have recovered.

Monkeys.—No experiments were made on monkeys with the Mvera Cattle strain.

Dogs.—It is curious that this strain, which is harmless to guinea-pigs and white rats, is the most virulent of the three to dogs, killing them, without exception, in about 30 days.

Guinea-pigs and White Rats.—The Mvera Cattle strain has no effect on guinea-pigs or white rats. It is important to note how different strains of *T. pecorum* vary in their behaviour to the smaller laboratory animals.

II. *The Wild-game Strain.*

Table XV.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Goats.		
1912.					
Jan. 22...	45	From Eland 44	11	70	Died of *T. pecorum.*
Feb. 7...	141	From Goat 45	8	54	" "
Mar. 16...	336	„ 45	9	48	" "
„ 16...	337	„ 45	9	20	" "
„ 16...	338	„ 45	9	41	" "
July 20...	899	From Reedbuck 912 ...	12	—	Still alive after 201 days.
„ 23...	898	From Hyæna 955	13	89	Died of *T. pecorum.*
„ 30...	978	From Eland 1013	13	93	" "
Aug. 11...	1037	From Waterbuck 1061	8	23	Mixed infection.
„ 18...	1035	From Bushbuck 1078...	18	—	Still alive after 172 days.
„ 18...	1037*a*	„ 1084...	22	23	Died of *T. pecorum.*
„ 19...	1106	From Oribi 1096.........	21	74	" "
„ 24...	1121	From Eland 1202	16	82	" "
Sept. 4...	1031	„ 1044	15	43	" "
„ 7...	1135	From Bushbuck 1261...	19	85	" "
„ 11...	1307	From Buffalo 1304......	26	35	" "
„ 17...	1383	From Koodoo 1380......	6	68	" "
Oct. 6...	1474	From Eland 1471	22	—	Still alive after 123 days.
		Average......	15	56	
			Monkeys.		
April 9...	403	From Dog 355............	9	236	Died of *T. pecorum.*
July 20...	913	From Reedbuck 912 ...	—	—	Never showed trypanosomes.
„ 23...	959	From Hyæna 955	—	—	" "
„ 30...	1014	From Eland 1013	27	101	Died of *T. pecorum.*
Aug. 11...	1062	From Waterbuck 1061	—	—	Never showed trypanosomes.
„ 18...	1079	From Bushbuck 1078...	—	—	" "
„ 18...	1085	„ 1084...	11	88	Died of *T. pecorum.*
„ 19...	1087	From Oribi 1096.........	—	—	Never showed trypanosomes.
Sept. 7...	1262	From Bushbuck 1261...	—	—	" "
„ 11...	1305	From Buffalo 1304......	—	—	" "
„ 17...	1381	From Koodoo 1380......	—	—	" "
„ 25...	1478	From Hartebeeste 1453	—	—	" "
		Average	15	142	

* Duration includes the days of incubation; it dates from day of infection.

Table XV—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Dogs.		
1912.					
Jan. 22...	46	From Eland 44	—	—	Never showed trypanosomes.
Feb. 7...	134	From Goat 45	8	35	Died of *T. pecorum*.
„ 7...	138	„ 45	—	—	Never showed trypanosomes.
Mar. 20...	355	„ 45	8	222	Died of *T. pecorum*.
„ 20...	356	„ 45	12	30	„ „
„ 20...	357	„ 45	—	—	Never showed trypanosomes.
May 4...	514	From Dog 355...........	12	20	Died of *T. pecorum*.
„ 4...	515	„ 355...........	9	20	„ „
July 20...	914	From Reedbuck 912 ...	—	—	Never showed trypanosomes.
„ 23...	963	From Hyæna 955	—	—	„ „
„ 30...	1015	From Eland 1013	15	83	Died of *T. pecorum*.
Aug. 11...	1063	From Waterbuck 1061	—	—	Never showed trypanosomes.
„ 18...	1080	From Bushbuck 1078...	—	—	„ „
„ 18...	1086	„ 1084...	8	156	Died of *T. pecorum*.
„ 19...	1098	From Oribi 1096.........	—	—	Never showed trypanosomes.
„ 27...	1204	From Eland 1202	27	—	Alive after 163 days.
Sept. 7...	1263	From Bushbuck 1261...	—	—	Never showed trypanosomes.
„ 11...	1306	From Buffalo 1304......	—	—	„ „
„ 17...	1382	From Koodoo 1380......	—	—	„ „
„ 25...	1455	From Hartebeeste 1453	8	24	Died of *T. pecorum*.
		Average	12	74	
			Guinea-pig.		
Mar. 20...	358	From Goat 45	—	—	Never showed trypanosomes.
			Rats.		
Mar. 20...	360	From Goat 45	—	—	Never showed trypanosomes.
„ 21...	361	„ 45	—	—	„ „
May 4...	510	From Dog 355...........	9	29	Died of *T. pecorum*.
„ 4...	511	„ 355...........	9	14	„ „
Oct. 28...	1518	„ 355...........	—	—	Never showed trypanosomes.
„ 28...	1519	„ 355...........	—	—	„ „
Nov. 15...	1518	From Monkey 403	10	30	Died of *T. pecorum*.
„ 15...	1519	„ 403	17	30	„ „
„ 16...	1608	From Goat 1135	16	45	„ „
		Average	12	30	

* Duration includes the days of incubation; it dates from day of infection.

Disease set up in Various Animals by T. pecorum, *Nyasaland—The Wild-game Strain.*

Cattle.—No cattle were available for experiment.

Goats.—As might be expected, *T. pecorum* from the blood of antelope behaves in the same way to goats as that from cattle. It has the same virulence, gives rise to the same symptoms and *post-mortem* appearances.

Monkeys.—The monkey is an animal not very susceptible to *T. pecorum.*

Blood taken from a wild animal and injected at the same time and under exactly the same conditions into a healthy goat, monkey, and dog, will often infect the first and last and fail to infect the monkey. In 10 experiments in which all the goats became infected, 8 of the 10 monkeys remained healthy. The disease is also much more chronic in the monkey than in the goat, the average duration of the disease in the former being 142 days, whereas in the latter it is only 56 days.

Dogs.—In comparison with the goat, the dog is also somewhat refractory to the Wild-game strain. In nine experiments all the goats became infected, while their companion dogs, injected with the same blood, remained healthy.

Guinea-pigs.—The one guinea-pig inoculated remained unaffected.

White Rats.—This strain is irregular in its action towards white rats. In nine experiments four remained healthy, although the five which did become infected died, on an average, in 30 days, which is moderately rapid for *T. pecorum.*

III. *The Wild* Glossina morsitans *Strain.*

Table XVI.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Cattle.		
1912. Feb. 3	108	Fly country	?	140	Mixed infection.
			Goats.		
Jan. 25	89	Wild flies	15	60	Died of *T. pecorum.*
Feb. 3	125	„	7	36	Mixed infection.
„ 8	175	„	20	88	„ „
„ 13	200	„	—	—	Never showed *T. pecorum.*
„ 19	247	„	21	30	Mixed infection.
„ 22	281	„	10	59	„ „
Mar. 6	273	„	6	47	„ „
„ 9	270	„	—	—	Never showed *T. pecorum.*
„ 18	343	„	11	34	Died of *T. pecorum.*
„ 23	363	„	12	43	Mixed infection.
„ 28	369	From Goat 109	21	—	Still alive after 315 days.
April 1	397	Wild flies	10	60	Mixed infection.
„ 3	408	„	11	40	Died of *T. pecorum.*
„ 10	410	„	—	—	Never showed *T. pecorum.*
„ 15	414	„	10	36	Mixed infection.
„ 18	428	„	—	—	Never showed *T. pecorum.*
„ 19	422	„	7	69	Mixed infection.
„ 23	420	„	—	—	Never showed *T. pecorum.*
„ 24	415	„	16	49	Mixed infection.
„ 29	429	„	12	43	„ „
„ 29	430	„	10	45	„ „

* Duration includes the days of incubation; it dates from day of infection.

Table XVI—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Goats—*continued.*		
1912.					
May 4...	434	Wild flies	9	—	Still alive after 277 days.
,, 9...	266	,,	8	71	Mixed infection.
,, 9...	269	,,	14	22	,, ,,
,, 15...	416	,,	5	19	,, ,,
,, 17...	553	,,	—	—	Never showed *T. pecorum.*
,, 24...	565	,,	7	56	Mixed infection.
,, 28...	564	,,	5	29	Died of *T. pecorum.*
,, 28...	568	,,	9	24	Mixed infection.
,, 31...	570	,,	4	64	,, ,,
June 1...	569	,,	5	36	,, ,,
,, 1...	571	,,	11	23	,, ,,
,, 2...	620	,,	—	—	Never showed *T. pecorum.*
,, 5...	622	,,	15?	34	Mixed infection.
,, 8...	627	,,	12	24	,, ,,
,, 10...	634	,,	9	—	Still alive after 241 days.
,, 10...	635	,,	13	63	Died of *T. pecorum.*
,, 14...	640	,,	18	60	,, ,,
,, 17...	645	,,	16	32	,, ,,
,, 25...	716	,,	10	64	Mixed infection.
July 5...	711	,,	20	106	Died of *T. pecorum.*
,, 9...	795	,,	10	40	,, ,,
Oct. 14...	1689	From Goat 1635	9	38	Mixed infection.
,, 28...	1516	Wild flies	8	31	,, ,,
,, 31...	1538	,,	8	24	,, ,,
Nov. 13...	1588	,,	9	—	Still alive after 85 days.
,, 18...	1613	,,	—	—	Never showed *T. pecorum.*
,, 27...	1638	,,	10	65	Mixed infection.
Dec. 5...	1667	,,	11	—	Still alive after 63 days.
,, 6...	1676	,,	4	41	Mixed infection.
,, 11...	1685	,,	6	38	,, ,,
,, 14...	1693	From Goat 1634	9	—	Still alive after 54 days.
,, 19...	1703	Wild flies	7	—	,, ,, 49 ,,
		Average	11	46	
			Pigs.		
Nov. 18...	1611	Wild flies	—	—	Never showed *T. pecorum.*
,, 25...	1636	,,	13	—	Still alive after 73 days.
Dec. 3...	1665	,,	6	17	Mixed infection.
,, 6...	1674	,,	3	10	,, ,,
,, 11...	1683	,,	—	—	Never showed *T. pecorum.*
,, 16...	1701	,,	6	12	Mixed infection.
1913.					
Jan. 1...	1725	,,	6	21	Died of *T. pecorum.*
,, 16...	1771	From Dog 1702	14	—	Still alive after 21 days.
,, 21...	1781	Wild flies	6	—	,, ,, 16 ,,
		Average	8	15	
			Monkeys.		
1912.					
Jan. 24...	55	Wild flies	—	—	Never showed *T. pecorum.*
Feb. 8...	58	,,	—	—	,, ,,
,, 13...	219	,,	7	9	Mixed infection.
,, 20...	49	,,	—	—	Never showed *T. pecorum.*

* Duration includes the days of incubation; it dates from day of infection.

Table XVI—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
		Monkeys—*continued.*			
1912.					
April 10...	404	Wild flies	—	—	Never showed *T. pecorum.*
„ 15...	405	„	—	—	„ „
„ 18...	447	„	—	—	„ „
„ 18...	448	„	—	—	„ „
„ 19...	450	From Dog 328	24	202	Died of *T. pecorum.*
„ 19...	451	„ 328	17	165	„ „
„ 23...	465	Wild flies	—	—	Never showed *T. pecorum.*
„ 23...	467	„	24	188	Died of *T. pecorum.*
„ 26...	488	„	—	—	Never showed *T. pecorum.*
„ 27...	495	„	—	—	„ „
May 3...	504	„	7	11	Mixed infection.
„ 3...	505	„	—	—	Never showed *T. pecorum.*
„ 8...	523	„	—	—	„ „
„ 9...	521	„	—	—	„ „
„ 14...	545	„	—	—	„ „
„ 29...	596	„	34	150	Died of *T. pecorum.*
„ 31...	601	„	—	—	Never showed *T. pecorum.*
June 6...	623	„	24	180	Died of *T. pecorum.*
„ 7...	625	„	—	—	Never showed *T. pecorum.*
„ 11...	629	„	—	—	„ „
„ 18...	702	„	—	—	„ „
„ 25...	739	„	—	—	„ „
July 3...	788	„	—	—	„ „
Sept. 25...	1452	„	21	34	Died of *T. pecorum.*
Oct. 29...	1536	„	—	—	Never showed *T. pecorum.*
Nov. 11...	1586	„	—	—	„ „
		Average......	20	117	
		Dogs.			
Feb. 24...	258	From Monkey 219	12	43	Died of *T. pecorum.*
„ 28...	256	From Goat 89............	15	24	„ „
Mar. 9...	321	„ 125............	12	34	„ „
„ 9...	322	„ 125............	9	48	„ „
„ 9...	323	„ 89............	12	26	„ „
„ 9...	324	„ 89............	12	24	„ „
„ 9...	328	Wild flies	23	39	„ „
„ 17...	341	„	15	28	„ „
„ 20...	359	From Dog 258............	8	14	„ „
„ 22...	362	Wild flies	10	17	„ „
April 3...	400	„	16	18	„ „
„ 12...	436	„	8	37	Mixed infection.
„ 17...	446	„	33	60	Died of *T. pecorum.*
„ 20...	463	„	21	57	„ „
„ 20...	464	„	13	57	„ „
„ 24...	478	„	18	56	„ „
„ 25...	466	„	15	50	„ „
„ 25...	468	„	11	21	„ „
„ 29...	489	„	15	49	„ „
„ 30...	496	„	12	46	„ „
May 6...	506	„	17	41	„ „
„ 6...	507	„	19	42	„ „
„ 8...	525	„	—	—	Never showed *T. pecorum.*
„ 10...	522	„	18	38	Died of *T. pecorum.*
„ 17...	549	From Monkey 523	10	51	Mixed infection.

* Duration includes the days of incubation; it dates from day of infection.

Table XVI—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
		Dogs—*continued.*			
1912.					
May 17	551	Wild flies	8	25	Mixed infection.
„ 18	548	„	30	—	Still alive after 258 days.
„ 24	578	„	18	24	Died of *T. pecorum.*
„ 28	574	„	15	33	„ „
„ 29	595	„	3	22	Mixed infection.
„ 29	599	„	22	152	Died of *T. pecorum.*
„ 31	597	„	8	30	„ „
„ 31	602	„	5	—	Still alive after 175 days.
June 2	619	„	10	28	Died of *T. pecorum.*
„ 7	624	„	17	29	„ „
„ 8	626	„	8	54	Mixed infection.
„ 9	630	„	13	21	Died of *T. pecorum.*
„ 13	666	„	12	—	Still alive after 231 days.
„ 19	701	„	11	19	Died of *T. pecorum.*
„ 25	729	„	—	—	Never showed *T. pecorum.*
July 4	734	„	14	—	Still alive after 210 days.
Oct. 29	1537	„	—	—	Never showed *T. pecorum.*
Nov. 12	1587	„	20	58	Died of *T. pecorum.*
„ 19	1612	„	17	—	Still alive after 78 days.
„ 19	1615	From Goat 1610	9	17	Died of *T. pecorum.*
„ 25	1637	Wild flies	—	—	Never showed *T. pecorum.*
Dec. 4	1666	„	12	32	Died of *T. pecorum.*
„ 6	1675	„	—	—	Never showed *T. pecorum.*
„ 11	1684	„	—	—	„ „
„ 14	1691	From Goat 1634	9	21	Mixed infection.
„ 14	1695	„ 1635	5	16	„ „
„ 18	1702	Wild flies	16	33	Died of *T. pecorum.*
		Average	14	37	
		Guinea-pigs.			
April 19	452	From Dog 328	13	42	Died of *T. pecorum.*
June 14	672	„ 602	—	—	Never showed *T. pecorum.*
„ 14	673	„ 602	24	38	Died of *T. pecorum.*
„ 14	674	„ 547	—	—	Never showed *T. pecorum.*
„ 14	675	„ 547	24	44	Died of *T. pecorum.*
„ 14	676	„ 549	—	—	Never showed *T. pecorum.*
„ 14	677	„ 549	—	—	„ „
„ 14	680	„ 551	13	39	Mixed infection.
„ 14	681	„ 551	20	42	„ „
„ 14	682	„ 595	—	—	Never showed *T. pecorum.*
„ 14	683	„ 595	—	—	„ „
		Average	19	41	
		Rats.			
April 19	454	From Dog 328	6	28	Died of *T. pecorum.*
„ 19	455	„ 328	11	30	„ „
June 11	657	„ 551	13	31	Mixed infection.
„ 11	660	„ 602	5	70	„ „
„ 12	655	„ 549	—	—	Never showed *T. pecorum.*
„ 14	658	„ 595	—	—	„ „
Nov. 19	1616	„ 1610	—	—	„ „
Dec. 14	1692	„ 1634	9	27	Mixed infection.
„ 14	1696	„ 1635	—	—	Never showed *T. pecorum.*
		Average	9	37	

* Duration includes the days of incubation; it dates from day of infection.

Disease set up in Various Animals by T. pecorum, *Nyasaland—The Wild* G. morsitans *Strain.*

Cattle.—Only one ox was experimented with. It was sent into the "fly-country," and as a result incurred a double infection of *T. pecorum* and *T. capræ*, and died in 140 days.

Goats.—The wild flies brought up from the "low-country" were fed, as a rule, on a goat, monkey, and dog. As the flies were often infected with several species of pathogenic trypanosomes, the result was many cases of mixed infection. In 50 experiments with goats, 10, or one-fifth only, were infections with *T. pecorum* alone. As *T. pecorum* is the most rapidly fatal trypanosome to goats among the various kinds of trypanosomes found in this locality, it may be assumed that the 28 goats which died of mixed infection owed this mainly to the *T. pecorum* infection.

Pig.—In eight feeding experiments with wild *G. morsitans*, only two showed a pure infection with *T. pecorum.* One died in 21 days and the other is still alive after 16 days. Another pig was inoculated from Dog 1702 and is still alive after 21 days. This species of domestic animal may, therefore, be said to be susceptible to this disease.

Monkey.—To this strain as to the Wild-game strain the monkey shows its refractoriness. These animals had the same chance of taking the disease as the goats and the dogs, but in 28 experiments the monkeys only became infected six times (21·4 per cent.), whereas in 50 goats there were 42 infections (84 per cent.), and in 41 dogs 35 (85·4 per cent.). The duration of the disease in the monkey is also long, the average in four cases being 138 days.

Dogs.—The Wild *G. morsitans* strain is fatal to dogs, killing them, on an average, in 41 days. In 35 cases of this disease among dogs, only five have survived to the present date. One of these may be said to have recovered, as the trypanosomes disappeared from the blood and the blood ceased to be infective. The other four still show trypanosomes in the blood after 258, 231, 210, and 78 days respectively. The dog may, therefore, be said to be as susceptible to the Wild *G. morsitans* strain of *T. pecorum* as the goat, few escaping the disease when bitten by an infective fly.

Guinea-pigs and White Rats.—The susceptibility of these animals to the Wild *G. morsitans* strain would be considered by some to go to prove that exaltation of virulence obtains after passage through the fly. This, however, is doubtful. Passage through the fly would more probably restore the trypanosome to its normal form—sometimes increasing, sometimes decreasing, its virulence for a particular animal.

Comparison of the Three Strains in regard to their Virulence towards Various Animals.

Table XVII.—The Average Duration of the Disease in Various Animals, in days. The letter R means that the animal is refractory, that is, not susceptible to the disease.

Strain.	Donkey.	Ox.	Goat.	Pig.	Monkey.	Dog.	Guinea-pig.	White rat.
Mvera Cattle	87 ?	103 ?	64			34	R	R
Wild Game			56		142	74	R	30
Wild *G. morsitans*		140	46	21	117	37	41	37

On the whole it may be concluded that the Wild *G. morsitans* strain is the most virulent.

Table XVIII.—The Percentages of Recoveries in Various Animals from the Three Strains. The letter R stands for refractory.

Strain.	Donkey.	Ox.	Goat.	Pig.	Monkey.	Dog.	Guinea-pig.	White rat.
Mvera Cattle	80	37	14			0	R	R
Wild Game			17		0	0	R	0
Wild *G. morsitans*		0	6		0	2	0	0

It must be confessed that the margin of error in a table such as this must be enormous; the fallacies are many. It is difficult or impossible to say when or if an animal has recovered from a trypanosome disease. The numbers employed are often small. For example, there is 0 per cent. indicated in regard to Guinea-pig, Wild game strain. This animal is returned as refractory, but there was only one experiment. It is evident that it would be more satisfactory if, say, a minimum of 10 animals of each species was used in studying each different strain. But in the wilds of Nyasaland this is a counsel of perfection impossible to attain. At the present time the Commission finds the greatest difficulty in obtaining small numbers of such local animals as goats, monkeys, and dogs, while as to the smaller laboratory animals, they must come from England. This entails a heavy mortality, as may be judged from the fact that of the last consignment of 25 guinea-pigs and 50 rats only 9 of the former and 12 of the latter arrived at Kasu alive.

The Average Duration of Life, in days, of Various Animals infected by T. pecorum, Nyasaland.

The following table combines the results obtained from a study of the susceptibility of various animals to each of the three strains, so as to arrive at a numerical expression of the virulence of the *T. pecorum* of Nyasaland. It must be carefully noted that only fatal cases are taken into account. In human medicine duration of disease means the number of days between the beginning of the illness and recovery. Here it means the number of days between infection and death. The table also shows against which animal the energies of *T. pecorum* are chiefly directed. It is certain that it is only by the detailed study of many strains of each of the African species of trypanosomes that a satisfactory classification of this important group of hæmatozoa will be arrived at. This work must be done on the spot, where the natural conditions for the normal life of the parasite are present, and where frequent opportunity of passing through the invertebrate host—the tsetse fly—exists. It is absurd to expect to arrive at any classification at all approaching a true one by the study of strains of trypanosomes kept for many years and undergoing many vicissitudes in our European laboratories.

Table XIX.—The Average Duration of Life, in days, of Various Animals infected by *T. pecorum*.

	Donkey.	Cattle.	Goat.	Pig.	Monkey.	Dog.	Guinea-pig.	White rat.
Average duration, in days	87 ?	121 ?	55	21	129	48	41	33
Number of animals employed	1	4	59	1	11	57	5	10

Table XX.—The Percentages of Recoveries in Various Animals from *T. pecorum* Infection.

	Donkey.	Cattle.	Goat.	Pig.	Monkey.	Dog.	Guinea-pig.	White rat.
Percentages	80	35	12	0	0	1	0	0
Number of animals employed	5	17	70	1	11	63	5	10

THE CARRIER OF T. PECORUM, NYASALAND.

In Nyasaland the carrier is *G. morsitans*, of which 4·6 per 1000 were found infected by this species. This is the minimum, but the real proportion of infected flies is probably three or four times as great. An account of the development of *T. pecorum* in this tsetse fly will be dealt with in a future paper. There is also some evidence derived from the outbreak among the Mvera cattle that, given infected animals in a herd, it is possible that *Tabanidæ*, *Hæmatopota*, or other biting flies may act as mechanical carriers. The evidence that *Stomoxys* plays a similar *rôle* is unsatisfactory.

CONCLUSIONS.

1. The Mvera Cattle strain, the Wild-game strain and the Wild *G. morsitans* strain belong to the same species of trypanosome, *T. pecorum*.

2. *T. pecorum*, Nyasaland, is identical with the species found and described in Uganda.

3. It is an important disease of domestic animals in Nyasaland, being destructive to donkeys, oxen, goats, pigs, and dogs.

4. Its carrier in this district is *G. morsitans*, about 2 per cent. probably of the local wild flies being naturally infected with *T. pecorum*.

5. Its reservoir is the wild game inhabiting "fly-country," 14·4 per cent. of which were found to be infected with this trypanosome. It is hardly to be doubted that 100 per cent. are, or have been, infected.

6. It is recommended that if infected animals are found in a herd they should be destroyed or segregated, as there is a danger of biting flies other than the tsetse spreading the disease in the herd by mechanical transmission.

Morphology of Various Strains of the Trypanosome causing Disease in Man in Nyasaland.—The Mzimba Strain.

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[PLATES 1–3.]

INTRODUCTION.

Up to the present time it has usually been considered that almost all the cases of Human trypanosome disease in man in Nyasaland have been confined to a small area. This, the so-called Sleeping-Sickness District, has been described in a former paper,* but it may be repeated here that it is the part of the "fly-country" lying along the western shore of Lake Nyasa,

* 'Roy. Soc. Proc.,' B, vol. 86, p. 274.

between S. lat. 13° 20′ and 13° 50′, and extending some twenty miles inland. Through the centre of this area a road runs from Domira Bay on the Lake into North-East Rhodesia. This road, until lately, was a principal highway between the coast and Central Africa. Dr. Aylmer May, the Principal Medical Officer of North-East Rhodesia, who lately visited Kasu, informed the Commission that it was along this trade-route that all the North-East Rhodesian cases of Human trypanosome disease have occurred. It is said that some 25,000 native porters passed along this road every year, and as they entered a *Glossina palpalis* area at the Congo end of their journey, it seemed at first natural to suspect that the disease was true Sleeping Sickness, and had spread from west to east along this trade-route. This suspicion was shown to be groundless by the discovery that the parasite causing the disease in North-East Rhodesia and Nyasaland is not *Trypanosoma gambiense,* but a distinct and separate species giving rise to a totally different disease. The question then arose as to whether this was an imported or indigenous disease. It has, therefore, been one of the objects of this Commission to determine whether the trypanosome causing Human trypanosome disease in Nyasaland is restricted to the game and "fly" of the Proclaimed Area, or if it extends to the north and south along the "fly-belt." If it is found to extend over all the "fly-area" in Nyasaland, then the disease is probably native to the soil and not an importation from Tanganyika or the Congo.

But it will be well at this point to lay down definitely the various opinions or theories at issue. These are three in number.

First, that the Human trypanosome disease of North-East Rhodesia and Nyasaland is caused by a specific trypanosome, *T. rhodesiense,* that the wild game and "fly" are heavily infected with it, and that *T. brucei,* or Nagana, is absent altogether. This is the theory held by one school.

Second, that the wild game and "fly" are heavily infected throughout these "fly-areas" by *T. brucei,* but that at certain places or foci another trypanosome, *T. rhodesiense,* occurs, which is pathogenic to man as well as the other animals. That these two species of trypanosomes are indistinguishable morphologically or by their action on animals, except that one is capable of infecting man and the other not. That the only way to separate them is by inoculating man: if the man reacts it is *T. rhodesiense,* if not, *T. brucei.*

Third, that *T. brucei*—a common trypanosome of wild game, whose distribution extends from Zululand to the Sudan—and *T. rhodesiense* are one and the same species of trypanosome, and that wherever wild game and *G. morsitans* are found there also will be found cases of trypanosome disease in man. That the cause of the sparsity of cases in man in these areas is due to the fact that man is more or less refractory to the trypanosome, and that it is only rarely that the "fly" meets with a susceptible subject. That this is the reason why the cases of Human trypanosome disease in the Luangwa valley and in Nyasaland do not tend to increase in numbers. The disease remains stationary, as it probably has done during the last thousand years. This is the working hypothesis held at present by this Commission.

These, then, are the points at issue, and it would appear at present that the only way of solving the problem will be by searching and finding out whether cases of *T. brucei* disease, or Nagana, in man occur wherever *G. morsitans* and this parasite are found together. Already cases have been found on the Rovuma river on the borders of German and Portuguese East Africa, and in the Hartley District south of the Zambesi, in Europeans and natives, who certainly could only have contracted the disease in these widely separated districts.

Thanks to Mr. Garden, the Government Veterinary Officer, the Commission have had the opportunity of studying a trypanosome of the Nagana type found in the blood of a donkey at Mzimba (lat. 11° 55′ S., long. 33° 35′ E.), about 100 miles north of the northern border of the Proclaimed Area. It is proposed, then, in this paper to describe this trypanosome, in pursuance of the policy of describing in detail as many strains as possible of this type of trypanosome, if peradventure some method of separating *T. brucei* from *T. rhodesiense* may be discovered, or of proving them to be one and the same species.

MORPHOLOGY OF THE MZIMBA STRAIN.

A. *Living, Unstained.*

The movements of this trypanosome in the living condition are similar to *T. brucei* and *T. gambiense* in being non-translatory.

B. *Fixed and Stained.*

The blood films were fixed, stained and measured as previously described in the "Proceedings."*

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* 'Roy. Soc. Proc.,' B, vol. 81, pp. 16 and 17.

Table I.—Measurements of the Length of the Trypanosome of the Mzimba Strain.

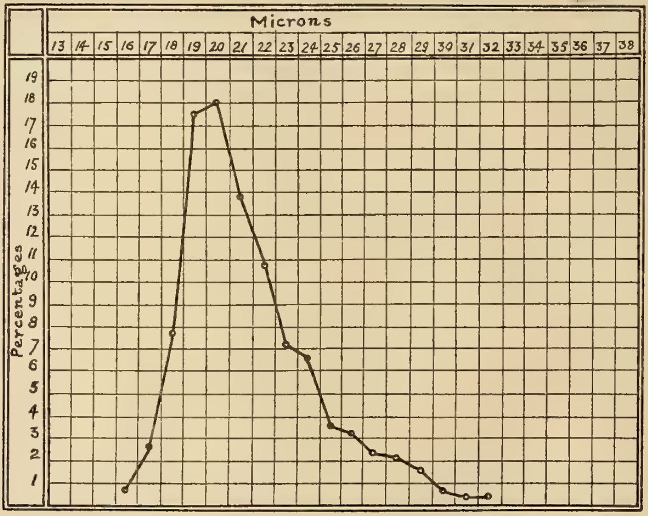
Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912.							
Mar. 28	365	Donkey	Osmic acid	Giemsa	18 ·3	21 ·0	16 ·0
" 2	384	Goat	"	"	19 ·8	26 ·0	18 ·0
" 27	368	"	"	"	20 ·1	23 ·0	17 ·0
Apr. 22	381	"	"	"	20 ·2	25 ·0	18 ·0
" 29	384	"	"	"	22 ·8	32 ·0	18 ·0
" 29	402	Monkey	"	"	21 ·2	30 ·0	17 ·0
May 6	402	"	"	"	21 ·6	29 ·0	18 ·0
Mar. 27	366	Dog	"	"	22 ·7	30 ·0	19 ·0
" 27	367	"	"	"	22 ·2	29 ·0	19 ·0
" 28	366	"	"	"	22 ·0	30 ·0	19 ·0
" 28	367	"	"	"	21 ·6	30 ·0	19 ·0
" 29	366	"	"	"	22 ·0	32 ·0	17 ·0
" 29	367	"	"	"	20 ·9	24 ·0	19 ·0
" 30	366	"	"	"	22 ·3	29 ·0	17 ·0
Apr. 1	366	"	"	"	20 ·2	24 ·0	17 ·0
" 4	366	"	"	"	20 ·9	25 ·0	16 ·0
" 4	366	"	"	"	21 ·6	26 ·0	17 ·0
" 8	366	"	"	"	21 ·8	31 ·0	19 ·0
" 8	366	"	"	"	20 ·3	27 ·0	17 ·0
" 8	387	"	"	"	23 ·1	29 ·0	17 ·0
" 8	388	"	"	"	19 ·6	23 ·0	18 ·0
" 11	366	"	"	"	22 ·0	31 ·0	18 ·0
" 11	388	"	"	"	19 ·6	21 ·0	18 ·0
" 15	387	"	"	"	22 ·0	30 ·0	18 ·0
" 15	388	"	"	"	20 ·6	25 ·0	16 ·0
May 9	512	Rat	"	"	23 ·3	31 ·0	19 ·0
" 9	512	"	"	"	24 ·3	29 ·0	19 ·0
" 9	513	"	"	"	22 ·6	28 ·0	16 ·0
" 9	513	"	"	"	21 ·8	26 ·0	16 ·0
" 10	512	"	"	"	21 ·2	26 ·0	19 ·0
" 10	512	"	"	"	21 ·1	27 ·0	18 ·0
" 10	513	"	"	"	20 ·9	25 ·0	18 ·0
" 10	513	"	"	"	20 ·3	23 ·0	17 ·0
" 11	512	"	"	"	22 ·1	28 ·0	18 ·0
" 11	512	"	"	"	21 ·0	27 ·0	18 ·0
" 11	513	"	"	"	20 ·2	24 ·0	17 ·0
" 11	513	"	"	"	22 ·2	30 ·0	18 ·0
" 13	512	"	"	"	20 ·4	25 ·0	18 ·0
" 13	512	"	"	"	20 ·2	26 ·0	18 ·0
" 13	513	"	"	"	19 ·4	26 ·0	17 ·0
" 13	513	"	"	"	19 ·3	23 ·0	17 ·0
" 14	512	"	"	"	21 ·7	28 ·0	17 ·0
" 14	512	"	"	"	21 ·5	28 ·0	17 ·0
" 14	513	"	"	"	21 ·7	28 ·0	19 ·0
" 14	513	"	"	"	22 ·5	29 ·0	19 ·0
" 15	512	"	"	"	24 ·1	30 ·0	17 ·0
" 15	512	"	"	"	24 ·5	32 ·0	18 ·0
" 15	513	"	"	"	21 ·2	26 ·0	19 ·0
" 15	513	"	"	"	21 ·6	28 ·0	18 ·0
" 16	513	"	"	"	21 ·2	31 ·0	18 ·0
					21 ·4	32 ·0	16 ·0

The average length of the trypanosome of the Mzimba strain, in different species of animals, taken from Table I, is as follows :—

Table II.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Donkey...............	20	18 ·3	21 ·0	16 ·0
Goat..................	80	20 ·7	32 ·0	17 ·0
Monkey	40	21 ·4	30 ·0	17 ·0
Dog	360	21 ·4	32 ·0	16 ·0
Rat	500	21 ·6	32 ·0	16 ·0

Chart 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of the Trypanosome of the Mzimba Strain.

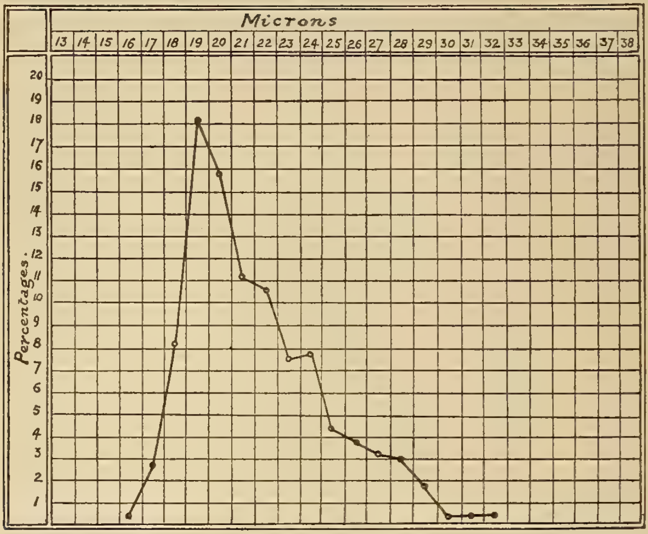


This curve is made up of measurements from 20 specimens of trypanosomes taken from the original donkey, 80 from the goat, 40 from the monkey, 360 from the dog, and 500 from the rat.

Table III.—Distribution in respect to Length of 1000 Individuals of the Trypanosome of the Mzimba Strain.

Animal.	In microns.																	Average length.
	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	
Donkey.........	3	1	6	7	2	1	—	—	—	—	—	—	—	—	—	—	—	18·3
Goat	—	—	5	6	4	2	1	1	—	—	1	—	—	—	—	—	—	19·8
”	—	1	—	5	8	3	2	1	—	—	—	—	—	—	—	—	—	20·1
”	—	—	4	5	4	2	2	1	1	1	—	—	—	—	—	—	—	20·2
”	—	—	1	2	4	2	2	2	1	3	—	—	1	1	—	—	1	22·8
Monkey	—	3	4	1	4	2	—	1	1	—	—	1	1	1	1	—	—	21·2
”	—	—	1	2	6	4	2	1	2	—	—	—	1	1	—	—	—	21·6
Dog	—	—	—	1	3	6	4	1	1	—	—	1	1	1	1	—	—	22·7
”	—	—	—	1	1	9	3	2	1	1	—	1	—	1	—	—	—	22·2
”	—	—	—	1	4	5	3	5	—	1	—	—	—	—	1	—	—	22·0
”	—	—	—	5	1	5	4	2	2	—	—	—	—	—	1	—	—	21·6
”	—	1	—	1	5	6	2	1	—	—	2	1	—	—	—	—	1	22·0
”	—	—	—	3	3	8	5	—	1	—	—	—	—	—	—	—	—	20·9
”	—	1	—	2	3	2	2	2	5	1	1	—	—	1	—	—	—	22·3
”	—	2	—	6	4	3	3	1	1	—	—	—	—	—	—	—	—	20·2
”	2	1	—	3	1	4	4	2	1	2	—	—	—	—	—	—	—	20·9
”	—	1	2	1	4	2	4	—	2	1	3	—	—	—	—	—	—	21·6
”	—	—	—	5	4	4	—	2	2	—	2	—	—	—	—	1	—	21·8
”	—	1	4	6	3	1	2	—	—	1	—	2	—	—	—	—	—	20·3
”	—	1	—	2	1	3	2	2	2	1	3	1	1	1	—	—	—	23·1
”	—	—	4	6	7	1	—	2	—	—	—	—	—	—	—	—	—	19·6
”	—	—	2	5	2	2	2	1	1	1	1	1	1	—	—	1	—	22·0
”	—	—	3	4	11	2	—	—	—	—	—	—	—	—	—	—	—	19·6
”	—	—	1	1	6	1	4	4	1	—	—	—	1	—	1	—	—	22·0
”	1	—	1	3	6	3	3	—	2	1	—	—	—	—	—	—	—	20·6
Rat	—	—	—	2	1	5	1	1	2	4	2	—	1	—	—	1	—	23·3
”	—	—	—	3	1	—	2	2	2	1	3	3	—	3	—	—	—	24·3
”	1	1	1	1	2	—	3	1	5	—	2	2	1	—	—	—	—	22·6
”	1	2	1	1	2	2	3	—	1	5	2	—	—	—	—	—	—	21·8
”	—	—	—	4	5	2	6	—	2	—	1	—	—	—	—	—	—	21·2
”	—	—	1	4	6	3	1	1	2	1	—	1	—	—	—	—	—	21·1
”	—	—	2	1	7	3	3	2	1	1	—	—	—	—	—	—	—	20·9
”	—	1	1	8	2	1	3	4	—	—	—	—	—	—	—	—	—	20·3
”	—	—	2	—	6	1	3	1	3	2	—	1	1	—	—	—	—	22·1
”	—	—	2	2	4	7	—	3	1	—	—	1	—	—	—	—	—	21·0
”	—	2	3	3	5	1	2	1	3	—	—	—	—	—	—	—	—	20·2
”	—	—	2	4	—	6	1	1	1	1	—	2	1	—	1	—	—	22·2
”	—	—	3	5	5	1	2	2	1	1	—	—	—	—	—	—	—	20·4
”	—	—	2	9	2	3	2	—	1	—	1	—	—	—	—	—	—	20·2
”	—	2	3	9	3	1	1	—	—	—	1	—	—	—	—	—	—	19·4
”	—	1	8	4	2	2	1	2	—	—	—	—	—	—	—	—	—	19·3
”	—	1	1	3	2	2	3	3	3	1	—	—	1	—	—	—	—	21·7
”	—	3	2	3	2	1	2	1	2	—	—	2	2	—	—	—	—	21·5
”	—	—	—	6	4	—	4	1	2	—	1	1	1	—	—	—	—	21·7
”	—	—	—	4	2	2	4	3	1	—	1	—	1	2	—	—	—	22·5
”	—	1	2	1	1	—	2	1	2	2	1	1	3	2	1	—	—	24·1
”	—	—	2	1	1	1	1	3	1	2	1	2	1	2	—	—	2	24·5
”	—	—	—	7	1	5	1	3	1	—	2	—	—	—	—	—	—	21·2
”	—	—	1	2	8	3	2	—	1	1	1	—	1	—	—	—	—	21·6
”	—	—	2	4	5	4	—	2	1	—	—	—	1	—	—	1	—	21·2
Total...........	8	27	79	175	180	139	109	72	66	36	32	24	22	16	7	4	4	
Percentages ...	0·8	2·7	7·9	17·5	18·0	13·9	10·9	7·2	6·6	3·6	3·2	2·4	2·2	1·6	0·7	0·4	0·4	

Chart 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of the Mzimba Strain, taken from Rats alone.



The similarity in the curve of the Mzimba strain and the curve of the Wild-game strain* is remarkable, and there can be little doubt that the same trypanosome is being dealt with. This is what might be expected, seeing there is probably only one animal between the wild *G. morsitans* and the experimental animal in both cases.

Table IV.—Measurements giving the Average Distance from the Posterior Extremity to Micronucleus, Micronucleus to Nucleus, etc., of 1000 Individuals of the Trypanosome of the Mzimba Strain.

Posterior extremity to micronucleus.	Micronucleus to nucleus.	Diameter of nucleus.†	Nucleus to anterior extremity.	Flagellum.
Short and Stumpy (16–21 microns), 608 Individuals.				
1·2	4·4	2·9	10·6	0·4
Intermediate (22–24 microns), 247 Individuals.				
1·5	5·3	3·0	10·4	2·6
Long and Slender (25–32 microns), 145 Individuals.				
1·8	5·8	3·0	10·9	5·5
Average 1·5	5·2	3·0	10·6	2·8

† These measurements are made along the long axis of the trypanosome, and therefore if an oval nucleus is lying transversely, the measurement given will not represent the greatest length of the nucleus.

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* 'Roy. Soc. Proc.,' B, vol. 86, p. 394.

Table IV agrees very closely with the same table given in the paper on the "Morphology of the Trypanosome causing Disease in Man in Nyasaland."* It is doubtful if this detailed method of measurement assists in putting trypanosomes in their proper places, but it may be that when more work has been done, something may emerge. There can be little doubt that morphology, in future, will play an important part—perhaps the most important part—in the classification of trypanosomes.

Breadth.—The average breadth is 3·45 microns (maximum 5·75, minimum 1·5). The short and stumpy average 3·7 (maximum 5·75, minimum 2), the intermediate 3·16 (maximum 5, minimum 1·5), and the long and slender 2·84 (maximum 4·25, minimum 1·75). The breadth of the widest part of the body of the 1000 trypanosomes is measured, including the undulating membrane.

Shape.—This trypanosome closely resembles in general appearance the various strains which have already been described from man, wild game and wild *G. morsitans.* Three black-and-white plates are given (Plates 1–3), which will give a better idea of the appearance of this strain than a written description.

Table V.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Mzimba Strain.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.	Proportion to all forms per 1000.
1912.				
Mar. 27 ...	367	Dog	0	0
„ 28 ...	367	„	10	60
„ 28 ...	366	„	3	18
„ 29 ...	367	„	8	48
„ 29 ...	366	„	13	78
„ 30 ...	366	„	12	72
Apr. 1 ...	366	„	7	42
„ 25 ...	387	„	15	90
„ 29 ...	387	„	25	150
May 2 ...	387	„	37	222
„ 10 ...	513	Rat	11	66
„ 10 ...	512	„	22	132
„ 11 ...	513	„	19	114
„ 11 ...	512	„	12	72
„ 13 ...	513	„	7	42
„ 13 ...	512	„	5	30
„ 14 ...	513	„	10	60
„ 15 ...	513	„	28	168
„ 16 ...	513	„	33	198
„ 16 ...	512	„	3	18
		Average	14·7	88

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* 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 428.

Micronucleus.—Situated, on an average, 1·8 microns from the posterior extremity in the long and slender, 1·5 in the intermediate, and 1·2 in the short and stumpy.

Undulating Membrane.—Well developed.

Flagellum.—The flagellum in the short and stumpy averages 0·4 micron (maximum 4, minimum 0·2), in the intermediate 2·6 (maximum 6, minimum 1) and in the long and slender 5·5 (maximum 10, minimum 2). Total average 2·8 (maximum 10, minimum 1).

Table VI.—Number of Flagellated and Non-flagellated Forms found among 1000 Trypanosomes of the Mzimba Strain.

Short and stumpy.			Intermediate.			Long and slender.		
Length, microns.	Non-flagellated.	Flagellated.	Length, microns.	Non-flagellated.	Flagellated.	Length, microns.	Non-flagellated.	Flagellated.
16	8	0	22	34	75	25	0	36
17	27	0	23	12	60	26	0	32
18	73	6	24	5	61	27	0	24
19	154	21				28	0	22
20	153	27				29	0	16
21	94	45				30	0	7
						31	0	4
						32	0	4
Totals	509	99		51	196		0	145

If the trypanosomes are divided into non-flagellar and flagellar, there are 56 per cent. of the former and 44 per cent. of the latter.

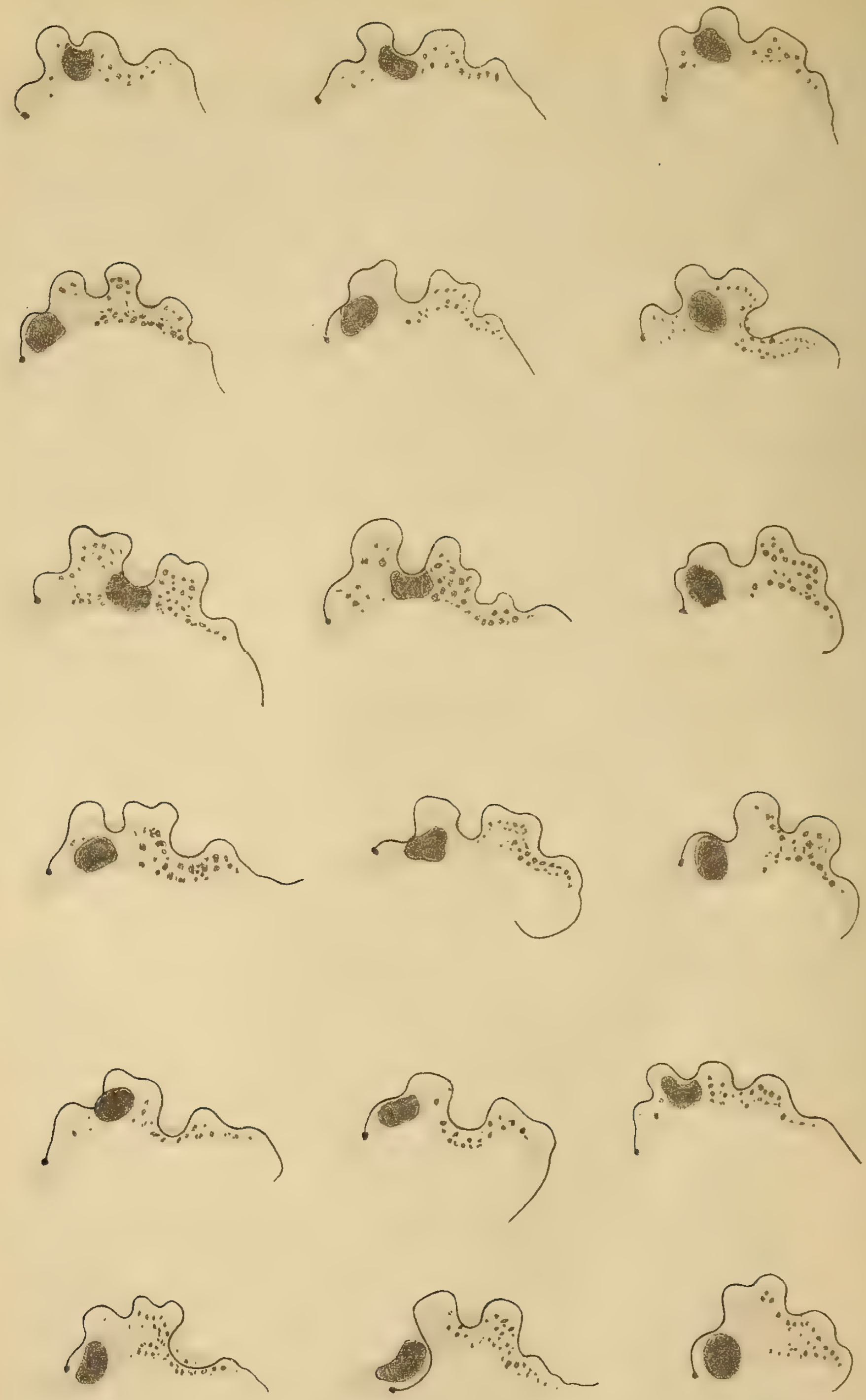
If we divide the 1000 Mzimba trypanosomes by length into short and stumpy (16 to 21 microns), intermediate (22 to 24 microns), and long and slender (25 to 32 microns), the percentages are 60·8, 24·7, and 14·4 respectively.

CONCLUSIONS.

1. The trypanosome of the Mzimba strain is the same species as that occurring in the wild game inhabiting the Proclaimed Area, Nyasaland.

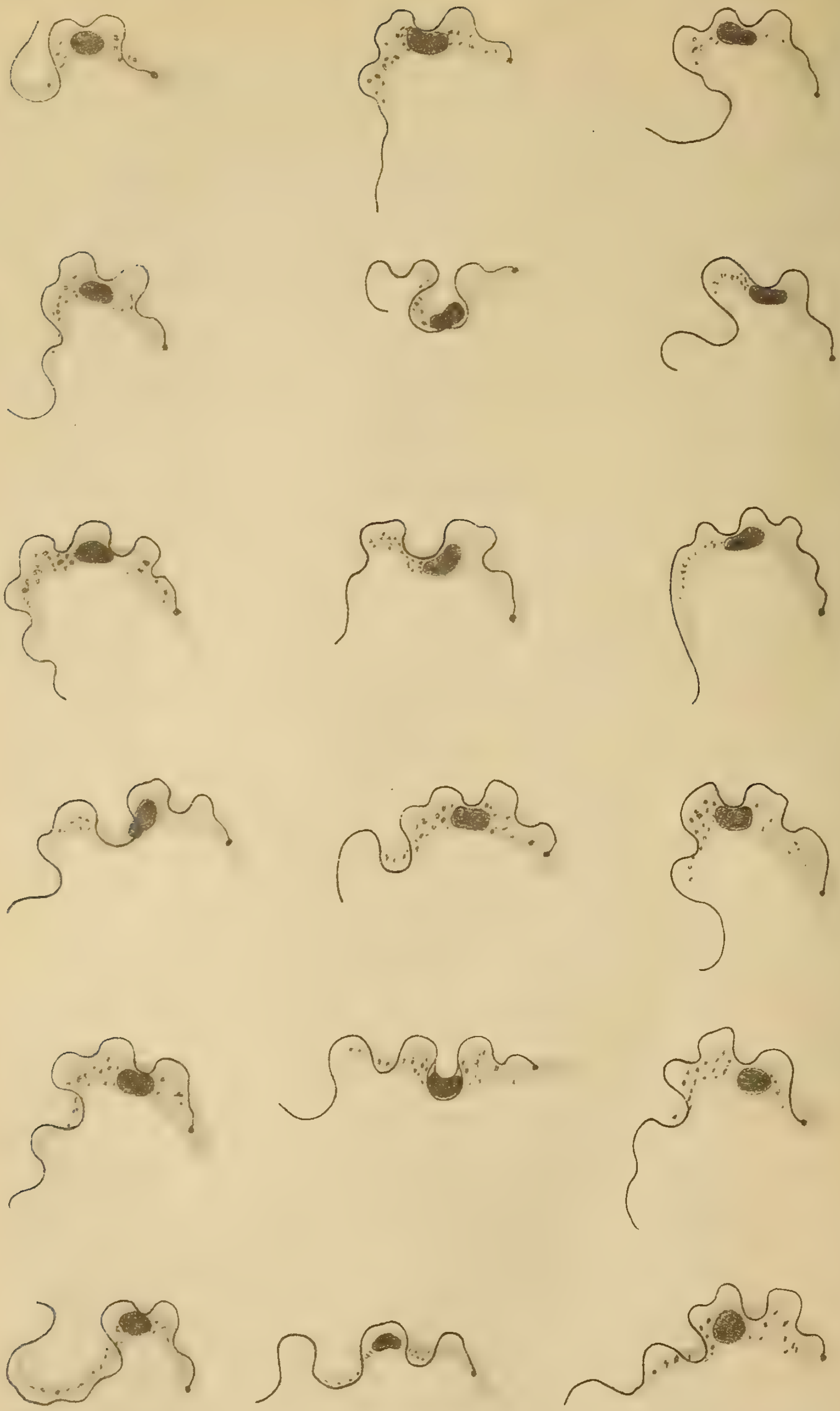
2. It has already been concluded that this species is *T. brucei* vel *rhodesiense*.

3. Hence it would appear that wild *G. morsitans* occurring in a district 100 miles north of the Proclaimed Area are infected with the trypanosome which causes the Human trypanosome disease of Nyasaland.



M. E. Bruce, del. Huth, Lith.r London.

Short & Stumpy

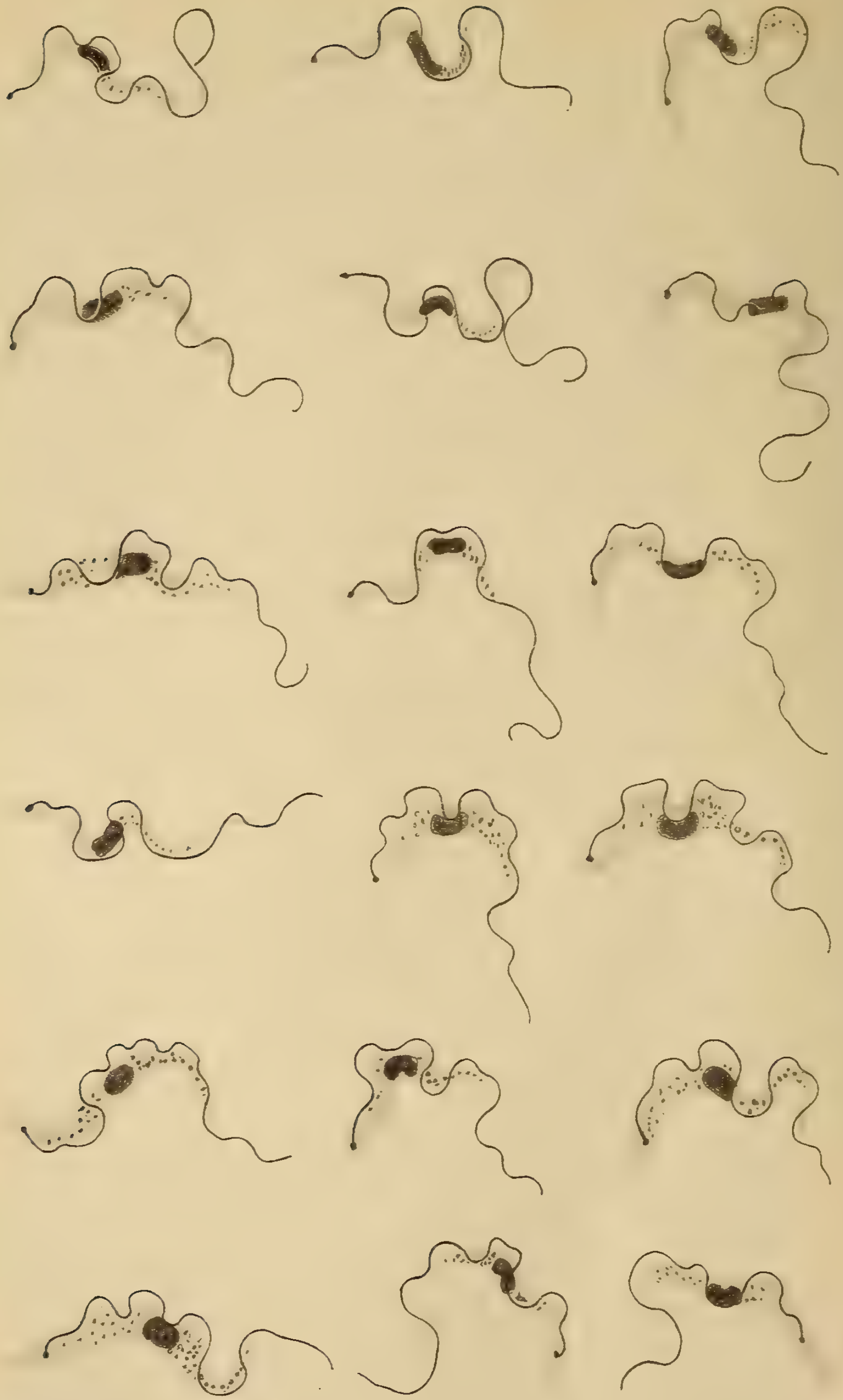


M.E. Bruce del.

Huth, Lith.rs London.

Intermediate

Bruce & others. Roy. Soc. Proc. B. vol. 87, Pl. 3.



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Long & Slender

DESCRIPTION OF PLATES.

Plate 1.

T. brucei vel *rhodesiense*. Short and stumpy forms, 16-21 microns in length. Stained Giemsa. × 2000.

Plate 2.

T. brucei vel *rhodesiense*. Intermediate forms, 22–24 microns in length. Stained Giemsa × 2000.

Plate 3.

T. brucei vel *rhodesiense*. Long and slender forms, 25–32 microns in length. Stained Giemsa. × 2000.

The Trypanosome causing Disease in Man in Nyasaland.—Susceptibility of Animals to the Human Strain.

By Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S.; Majors David Harvey and A. E. Hamerton, D.S.O., R.A.M.C.; and Lady Bruce, R.R.C.

(Scientific Commission of the Royal Society, Nyasaland, 1913.)

(Received May 26,—Read June 12, 1913.)

Introduction.

In previous papers the morphology of various strains of this trypanosome —from man, wild game and wild *Glossina morsitans*—was described, and the different strains compared.

This paper describes the action on animals of the five strains derived from cases of trypanosome disease in man in Nyasaland, and compares their virulence. In a future paper it is proposed to describe in the same way the action on animals of the Wild-game and Wild *G. morsitans* strains, but up to the present this has been impossible on account of the scarcity of experimental animals.

ANIMALS SUSCEPTIBLE TO THE TRYPANOSOME CAUSING DISEASE IN MAN IN NYASALAND. I.—THE HUMAN STRAIN.

I. *Strain I, Mkanyanga.*

Table I.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Cattle.		
1912.					
Feb. 7...	167	From Rat 36	—	—	Never showed trypanosomes.
,, 7...	168	,, 36	13	134	Died of Strain I.
April 23...	473	From Guinea-pig 373...	—	—	Never showed trypanosomes.
,, 23...	474	,, 373...	9	—	Still alive after 325 days.
			Goats.		
Feb. 7...	142	From Rat 36	8	33	Died of Strain I.
,, 7...	144	,, 36	8	19	,, ,,
,, 7...	146	,, 36	—	—	Never showed trypanosomes.
,, 7...	149	,, 36	22	27	Died of Strain I.
April 23...	411	From Guinea-pig 373...	9	55	,, ,,
,, 23...	417	,, 373...	6	25	,, ,,
,, 23...	418	,, 373...	44	45	,, ,,
		Average......	16·2	34·0	
			Sheep.		
Feb. 7...	162	From Rat 36	2	19	Died of Strain I.
,, 7...	163	,, 36	2	23	,, ,,
,, 7...	164	,, 36	2	33	,, ,,
April 23...	475	From Guinea-pig 373...	9	16	,, ,,
,, 23...	476	,, 373...	9	53	,, ,,
,, 23...	477	,, 373...	13	57	,, ,,
		Average......	6·2	33·5	
			Baboon.		
1911.					
Dec. 21...	12	From Monkey 3	—	—	Never showed trypanosomes.
			Monkeys.		
Nov. 16...	3	Mkanyanga	14?	35	Died of Strain I.
1912.					
Jan. 19...	24	From Guinea-pig 13 ...	11?	28	,, ,,
Feb. 16...	233	From Monkey 24	3	28	,, ,,
,, 16...	234	,, 24	3	16	,, ,,
April 23...	469	From Guinea-pig 373...	13	31	,, ,,
,, 23...	470	,, 373...	6	42	,, ,,
		Average......	6·2	30·0	

* Duration includes the days of incubation; it dates from day of infection.

Table I—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
		Dogs.			
1911.					
Oct. 15...	1	Mkanyanga...............	11 ?	46	Died of Strain I.
Nov. 16...	2	From Dog 1...............	14 ?	21	„ „
Dec. 7...	7	„ 2...............	8	20	„ „
„ 20...	11	„ 7...............	9	30	„ „
1912.					
Jan. 15...	14	„ 11...............	8	14	„ „
„ 31...	111	„ 14...............	6	14	„ „
		Average......	7·7	24·2	
		Rabbit.			
June 14...	671	From Sheep 476.........	12	42	Died of Strain I.
		Guinea-pigs.			
1911.					
Dec. 16...	5	From Rat 4...............	13 ?	13	Died of Strain I.
„ 21...	13	From Monkey 3.........	11	100	„ „
1912.					
Feb. 7...	165	From Rat 36	12	42	„ „
„ 7...	166	„ 36	15	36	„ „
Mar. 27...	372	From Guinea-pig 13 ...	19	72	„ „
„ 27...	373	„ 13 ...	15	—	Killed April 23.
		Average......	15·2	52·5	
		Rats.			
1911.					
Nov. 28...	4	From Dog 1...............	8	19	Died of Strain I.
Dec. 16...	8	From Rat 4...............	—	—	Never showed trypanosomes.
„ 16...	9	„ 4...............	?	13	Died of Strain I.
„ 29...	36	„ 9...............	25 ?	—	Killed.
„ 29...	37	„ 9...............	24 ?	26	Died of Strain I.
„ 29...	38	From Monkey 3.........	25 ?	33	„ „
1912.					
Feb. 16...	235	„ 24.........	4	25	„ „
„ 16...	236	„ 24.........	4	14	„ „
April 23...	471	From Guinea-pig 373...	7	15	„ „
„ 23...	472	„ 373...	6	22	„ „
Nov. 8...	1575	From Ox 474	—	—	Never showed trypanosomes.
		Average......	6·0	20·9	

* Duration includes the days of incubation; it dates from day of infection.

II. *Strain II, E——.*

Table II.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
		Cattle.			
1912.					
June 28...	764	From Dog 633............	—	—	Never showed trypanosomes.
„ 28...	765	„ 633............	—	—	„ „
„ 28...	766	„ 633............	27 ?	—	Shot November 20; broke leg.
		Goats.			
June 19...	643	From Dog 633............	12	52	Died of Strain II.
„ 19...	650	„ 633............	12	49	„ „
„ 19...	651	„ 633............	5	62	„ „
„ 19...	652	„ 633............	12	43	„ „
		Average......	10·2	51·5	
		Sheep.			
June 28...	761	From Dog 633............	10	87	Died of Strain II.
		Monkeys.			
June 26...	750	From Dog 633............	5	17	Died of Strain II.
„ 26...	751	„ 633............	5	27	„ „
„ 26...	752	„ 633............	5	11	„ „
„ 26...	753	„ 633............	8	10	„ „
		Average......	5·7	16·2	
		Dogs.			
May 29...	632	From E——	12 ?	30	Died of Strain II.
„ 29...	633	From E——	12 ?	33	„ „
June 18...	703	From Dog 633............	6	24	„ „
„ 18...	704	„ 633............	6	40	„ „
		Average......	6·0	31·8	
		Rabbits.			
June 28...	762	From Dog 633............	13	33	Died of Strain II.
„ 28...	763	„ 633............	17	33	„ „
		Average......	15·0	33·0	
		Guinea-pigs.			
June 19...	723	From Dog 633............	—	—	Never showed trypanosomes.
„ 19...	724	„ 633............	—	—	„ „
„ 19...	725	„ 633............	—	—	„ „
Sept. 7...	723	From Sheep 761.........	12	114	Died of Strain II.
„ 7...	724	„ 761.........	19	86	„ „
„ 7...	725	„ 761.........	19	84	„ „
		Average......	17·0	94·7	
		Rats.			
June 19...	726	From Dog 633............	5	43	Died of Strain II.
„ 19...	727	„ 633............	8	36	„ „
„ 19...	728	„ 633............	5	52	„ „
		Average......	6·0	43·7	

* Duration includes the days of incubation; it dates from day of infection.

III. *Strain III, Chituluka.*

Table III.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Cattle.		
1912. July 23...	967	From Dog 577............	20	—	Still alive after 234 days.
,, 23...	968	,, 577............	83	—	,, ,,
			Goats.		
July 23...	937	From Dog 577............	13	36	Died of Strain III.
,, 23...	938	,, 577............	9	19	,, ,,
,, 23...	939	,, 577............	9	41	,, ,,
,, 23...	940	,, 577............	6	29	,, ,,
		Average......	9·2	31·2	
			Monkeys.		
July 23...	941	From Dog 577............	6	29	Died of Strain III.
,, 23...	942	,, 577............	6	14	,, ,,
,, 23...	943	,, 577............	6	20	,, ,,
,, 23...	944	,, 577............	6	11	,, ,,
		Average......	6·0	18·5	
			Dogs.		
June 27...	577	Chituluka	7	78	Died of Strain III.
July 23...	945	From Dog 577............	9	31	,, ,,
,, 23...	946	,, 577............	6	36	,, ,,
,, 23...	947	,, 577............	9	33	,, ,,
,, 23...	948	,, 577............	9	33	,, ,,
		Average......	8·0	42·2	
			Guinea-pigs.		
July 23...	949	From Dog 577............	20	62	Died of Strain III.
,, 23...	950	,, 577............	20	51	,, ,,
,, 23...	951	,, 577............	9	73	
		Average......	16·3	62	
			Rats.		
July 23...	952	From Dog 577............	6	24	Died of Strain III.
,, 23...	953	,, 577............	9	20	,, ,,
,, 23...	954	,, 577............	6	21	,, ,,
		Average......	7·0	21·7	

* Duration includes the days of incubation; it dates from day of infection.

IV. *Strain IV, Chipochola.*

Table IV.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
		Cattle.			
1912.					
Sept. 13...	1319	From Dog 1260	—	—	Never showed trypanosomes.
„ 13...	1320	„ 1260	—	—	„ „
Nov. 8...	1319	From Rat 1338	20	—	Still alive after 126 days.
„ 8...	1320	„ 1338	—	—	Never showed trypanosomes.
		Goats.			
Sept. 13...	1321	From Dog 1260	10	35	Died of Strain IV.
„ 13...	1322	„ 1260	13	59	„ „
„ 13...	1323	„ 1260	6	22	„ „
„ 13...	1324	„ 1260	24	33	„ „
		Average......	13·2	37·2	
		Monkeys.			
Sept. 13...	1325	From Dog 1260	10	16	Died of Strain IV.
„ 13...	1326	„ 1260	10	50	„ „
„ 13...	1327	„ 1260	6	51	„ „
„ 13...	1328	„ 1260	—	—	Never showed trypanosomes.
		Average......	8·7	39·0	
		Dogs.			
Sept. 4...	1260	Chipochola	8	32	Died of Strain IV.
„ 13...	1329	From Dog 1260	6	27	„ „
„ 13...	1330	„ 1260	6	27	„ „
„ 13...	1331	„ 1260	6	38	„ „
„ 13...	1332	„ 1260	6	40	„ „
		Average......	6·4	32·8	
		Rabbits.			
Nov. 6...	1566	From Rat 1338	5	15	Died of Strain IV.
„ 6...	1567	„ 1338	5	16	„ „
		Average......	5	15·5	
		Guinea-pigs.			
Sept. 13...	1333	From Dog 1260	—	—	Never showed trypanosomes.
„ 13...	1334	„ 1260	—	—	„ „
„ 13...	1335	„ 1260	—	—	„ „
Oct. 17...	1333	„ 1331	11	43	Died of Strain IV.
„ 17...	1334	„ 1331	11	70	„ „
„ 17...	1335	„ 1331	11	59	„ „
		Average......	11·0	57·3	
		Rats.			
Sept. 13...	1336	From Dog 1260	6	26	Died of Strain IV.
„ 13...	1337	„ 1260	6	28	„ „
„ 13...	1338	„ 1260	6	93	„ „
		Average......	6·0	49·0	

* Duration includes the days of incubation; it dates from day of infection.

V. *Strain V, Chibibi.*

Table V.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Goats.		
1912. Nov. 27...	1643	From Dog 1599	8	64	Died of Strain V.
,, 27...	1644	,, 1599	8	72	,, ,,
,, 27...	1645	,, 1599	5	41	,, ,,
,, 27...	1646	,, 1599	5	64	,, ,,
		Average......	6·5	60·2	
			Monkeys.		
Nov. 27...	1647	From Dog 1599	8	11	Died of Strain V.
,, 27...	1648	,, 1599	—	—	Never showed trypanosomes.
,, 27...	1649	,, 1599	5	21	Died of Strain V.
,, 27...	1650	,, 1599	5	44	,, ,,
		Average......	6·0	25·3	
			Dogs.		
Nov. 14...	1599	Chibibi	11	44	Died of Strain V.
,, 27...	1651	From Dog 1599	8	46	,, ,,
,, 27...	1652	,, 1599	5	34	,, ,,
,, 27...	1653	,, 1599	8	45	,, ,,
,, 27...	1654	,, 1599	5	41	,, ,,
1913. Jan. 15...	1768	From Rat 1744	12	—	Killed January 31.
		Average......	8·2	42·0	
			Rabbits.		
1912. Nov. 27...	1655	From Dog 1599	8	33	Died of Strain V.
,, 27...	1656	,, 1599	8	23	,, ,,
		Average......	8·0	28·0	
			Guinea-pigs.		
Nov. 27...	1657	From Dog 1599	—	—	Never showed trypanosomes.
,, 27...	1658	,, 1599	36	102	Died of Strain V.
,, 27...	1659	,, 1599	—	—	Never showed trypanosomes.
1913. Jan. 31...	1657	,, 1768	—	—	,, ,,
Feb. 10...	1657	From Guinea-pig 1658	10	—	Still alive after 43 days.
,, 10...	1659	,, 1658	24	—	,, ,,
		Average......	23·3	—	

* Duration includes the days of incubation; it dates from day of infection.

Table V—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Rats.		
1912.					
Nov. 27...	1660	From Dog 1599	5	38	Died of Strain V.
„ 27...	1661	„ 1599	5	30	„ „
„ 27...	1662	„ 1599	8	39	„ „
1913.					
Jan. 8...	1744	From Guinea-pig 1658	6	—	Killed January 22.
„ 28...	1817	From Monkey 1733 ...	2	20	From Transmission Experiment 1723. Died of Strain V.
		Average......	5·2	31·8	

* Duration includes the days of incubation; it dates from day of infection.

Disease set up in Various Animals by the Trypanosome causing Disease in Man in Nyasaland.—I. *The Human Strain.*

Ox.—This trypanosome is not as deadly to oxen as *Trypanosoma pecorum*; 13 experiments in all were made on cattle with the Human strain. Only one of these died, after an illness lasting 134 days. Four took the disease and recovered, while the remaining eight showed themselves refractory to the injection of blood containing the parasites. One of the recovered animals has been kept under observation for 325 days, and its blood was inoculated into a rat without result. At the present time these so-called recovered animals appear sleek and fat and look healthy, presenting a contrast to the chronic *T. pecorum* infected cattle, which still remain in poor condition. The animal which died was much emaciated, anæmic, and had marked gelatinous infiltration of the connective tissue at the base of the heart and large vessels. The trypanosomes were never numerous in its blood, and in fact only showed six times in the four and a-half months of its illness at the bi-weekly blood examination.

Goat.—This is a fatal disease in goats; 22 were used for experimental purposes, and not one of them recovered. The duration of the disease, on an average, was 41·8 days (19 to 72). One of these animals showed swelling of the face, but none developed opacity of the cornea.

Sheep.—This trypanosome seems as fatal to sheep as goats, killing seven, on an average, in 41·1 days (16 to 87). Among these seven, œdema of the face was noted as a prominent symptom in three. No opacity of cornea developed in any of them.

Baboon.—Only one was inoculated and it proved resistant.

Monkey.—Twenty monkeys died, on an average, in 25·8 days (10 to 51). The trypanosomes were always present in the blood and were often numerous or very numerous. In none of the monkeys was œdema of the face or corneal opacity noted.

Dog.—Twenty-five dogs were inoculated. All died, on an average, in 34·3 days (14 to 78). The parasites were always present on microscopical examination of the blood, and were often numerous and very numerous. In eight of the 25 dogs, opacity of the cornea and swelling of the face were present.

Rabbit.—Seven rabbits died, on an average, in 27·9 days (15 to 42). A rabbit suffering from this disease presents exactly the same clinical picture as that seen in rabbits suffering from Nagana. There is first swelling round the eyes ; then the face puffs up, and sores break out round the nose and the eyes. Next there is thickening of the ears, which eventually also become covered with sores exuding a serous fluid. Towards the end the eyes are completely closed up, the nose much swollen, and both eyes and nose discharge a purulent fluid.

Guinea-pig.—These animals are more refractory than rabbits, and often require to be re-inoculated before they take the disease ; 15 were used. They died, on an average, in 66·6 days (13 to 114). No prominent symptom, such as œdema or corneal opacity, was seen.

Rat.—Twenty-one were inoculated and died, on an average, in 30·3 days (13 to 93), with their blood swarming with trypanosomes and their spleens enormously enlarged.

Comparison of the Five Human Strains of the Trypanosome causing Disease in Man in Nyasaland, in regard to their Virulence towards Various Animals.

Table VI.—The Average Duration, in Days, of the Disease in Various Animals. The letter R means that the animal is refractory, that is, not susceptible to the disease.

Strain.	Ox.	Goat and sheep.	Baboon.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
I......	134	34	R	30	24	42	52	21
II......		59		16	32	33	95	44
III......		31		18	42		62	22
IV......		37		39	33	15	57	49
V......		60		25	42	28	102	32

There would appear to be little difference in the virulence of the five Human strains. If any distinction be made, it might be said that probably Strain I is the most and Strain V the least virulent.

Table VII.—The Percentages of Recoveries in Various Animals from the Five Human Strains. The letter R stands for refractory.

Strain.	Man.	Ox.	Goat and sheep.	Baboon.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
I......	0	50	0	R	0	0	0	0	0
II......	0	?	0		0	0	0	0	0
III......	0	100	0		0	0		0	0
IV......	0	100	0		0	0	0	0	0
V......	0		0		0	0	0	0	0

It would appear to be equally impossible to separate the Human strains by the percentages of recoveries. All the experimental animals, except the ox, succumb to the disease if once the parasite has obtained a footing.

Table VIII.—The Average Duration of Life, in Days, of Various Animals infected with the Human Strain. The letter R stands for refractory.

	Ox.	Goat and sheep.	Baboon.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Average duration, in days	134	42	R	26	34	28	67	30
No. of animals employed	1	29	1	20	25	7	15	21

This table shows the extreme virulence of this trypanosome for most animals except the ox. The guinea-pig is somewhat refractory, and often resists the first injection of infected blood, but not the second.

Table IX.—The Percentages of Recoveries in Various Animals infected with the Five Human Strains. The letter R stands for refractory.

	Man.	Ox.	Goat and sheep.	Baboon.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Percentages ...	0	80	0	R	0	0	0	0	0
No. of animals employed	5	5	29	1	20	25	7	15	21

CONCLUSIONS.

1. The trypanosome causing disease in man in Nyasaland is fatal to goats, sheep, dogs, and the smaller laboratory animals, killing them, without exception, in a few weeks. It is less virulent to cattle, many of which evidently escape.

2. No difference in virulence can be made out in these five Human strains.

3. It is not satisfactorily proved yet to what species this trypanosome belongs, but the Commission at present leans to the opinion that it is *T. brucei* (Plimmer and Bradford).

Plasmodium cephalophi, *sp. nov.*

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Majors DAVID HARVEY and A. E. HAMERTON, D.S.O., R.A.M.C.; and Lady Bruce, R.R.C.

(Scientific Commission of the Royal Society, Nyasaland, 1913.)

(Received May 26,—Read June 12, 1913.)

[PLATES 4 AND 5.]

It would appear from a perusal of the available literature that malaria of antelopes has not hitherto been described; it is therefore proposed to place on record the discovery of a plasmodium found in the blood of two of these animals in Nyasaland.

This parasite was first seen in the blood of a young duiker (*Cephalophus grimmi*), and was subsequently discovered in another young animal of the same species. Both these small antelope were at the time in captivity, and it was therefore possible to examine slides from day to day, and by this means a large number of parasites at various stages of development were observed, and some of these are figured in Plates 4 and 5.

The acute attack in one antelope, however, only lasted four days, and the parasites soon disappeared entirely from the peripheral blood, whereas in the other only a few parasites were seen, and these have persisted in the blood for some months.

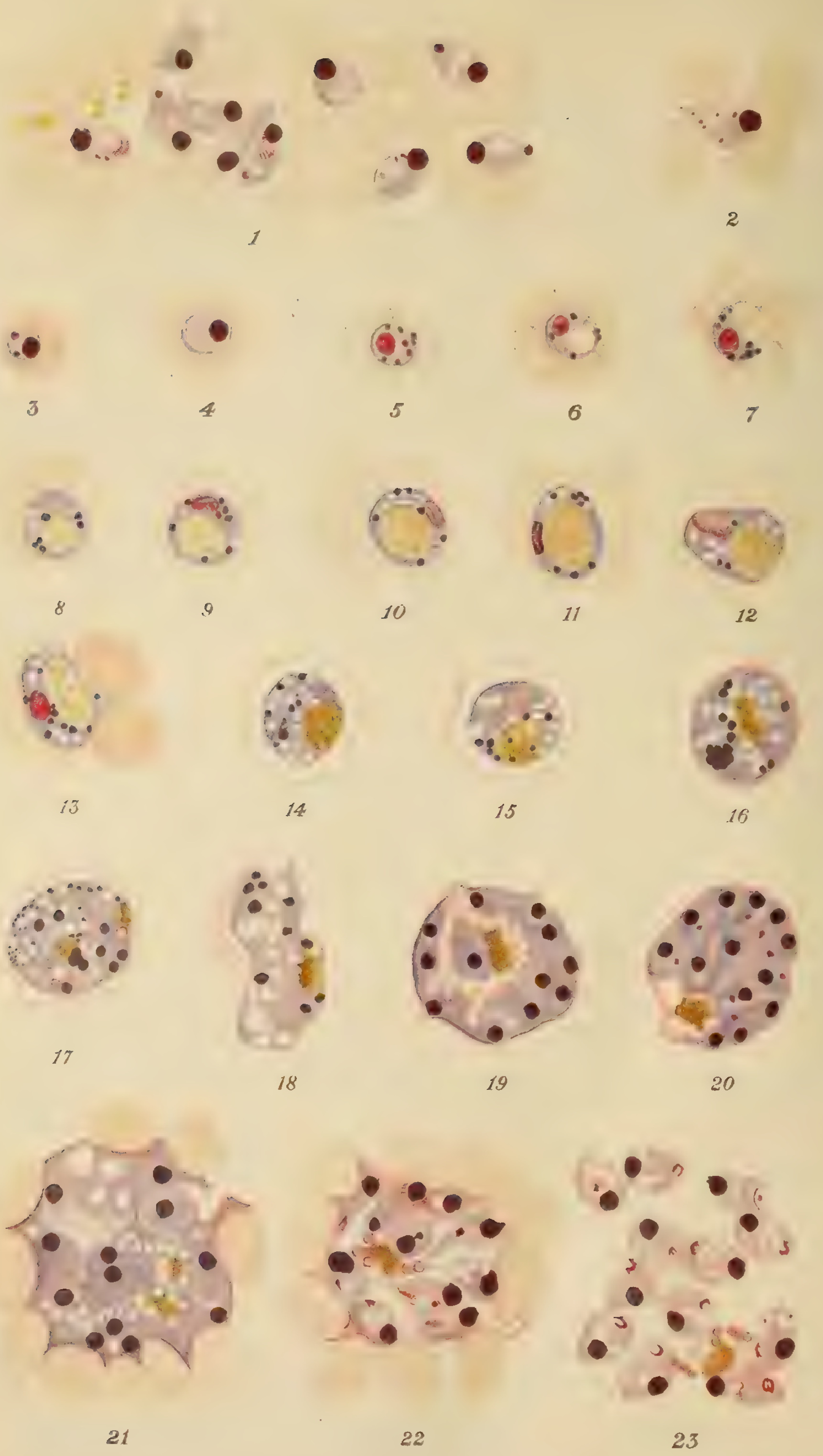
The parasites resemble somewhat *Plasmodium malariæ* of man, in that the gametocytes are circular and the schizonts have from eight to twelve merozoites; also amœboid movement is sluggish. They differ, however, in the marked enlargement and paleness of the red cell and in the arrangement and appearance of the pigment, which is not scattered throughout the parasite, but is collected in a single mass, and is pale yellow in colour (Plate 5). Schüffner's dots have never been seen, but in some instances (Plate 4, figs. 5, 6, and 7) pseudopodia were seen with a scar in the red cell recalling the formation of Maurer's dots in *Laverania malariæ* of man. The forms of trophozoite shown in Plate 4, figs. 6 to 12, are, so far as our experience goes, unique, first in the dense blue-staining (Giemsa) of the protoplasm, and, secondly, the great enlargement and marked paleness of the red cell with absence of Schüffner's dots, and the intense concentration of the portion of red cell in the food vacuole. This concentration, as will be seen in the figures, proceeds until nothing is left but a block of gamboge-yellow pigment (Plate 4, figs. 14 to 16). The distended red cells are so delicate that they are frequently ruptured in making the film, as is shown in Plate 5, figs. 7 and 8. The great size of the parasite is also remarkable, some of the free merozoites measuring 4 microns by 3·5 microns, the exact size of a normal red cell of the antelope. A full grown schizont, before cleavage of the protoplasm, measures 10 microns by 10 microns (Plate 4, fig. 19).

The presence in the gametocytes of numerous deeply staining chromatin granules, in addition to the faintly stained nucleus, is also remarkable. The arrangement of these granules in some of the merozoites suggests a relationship with the *Hæmoflagellata* (Plate 4, figs. 3 and 23); and in this connection it may be mentioned that one duiker was also naturally infected with a trypanosome of non-pathogenic type, which it is proposed to describe in a future paper.

In films taken some days and even months after the acute attack, the forms shown in Plate 5, figs. 12 to 16, are common, and but for the presence of pigment might be mistaken for faintly stained leucocytes. As these are the most persistent forms (macrogametocytes), attention is directed to them, as they are the forms likely to be met with in examination of blood films of antelope taken in the field.

One of the small duikers appeared out of condition for a few days, while the parasites were numerous in the blood: coat staring, nose hot and dry; but it soon regained its health, and has remained healthy since. The other duiker was apparently not at all affected, although a few parasites could be found in its blood for some six months.

The duiker which was chronically infected was captured in the low country near the Lake shore, and was about three months old when brought to Kasu Hill, where a few weeks later a few parasites were found in its blood. It is therefore probable that it was infected before capture. Whereas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Plasmodium cephalophi sp. nov.

M. E. Bruce, del.

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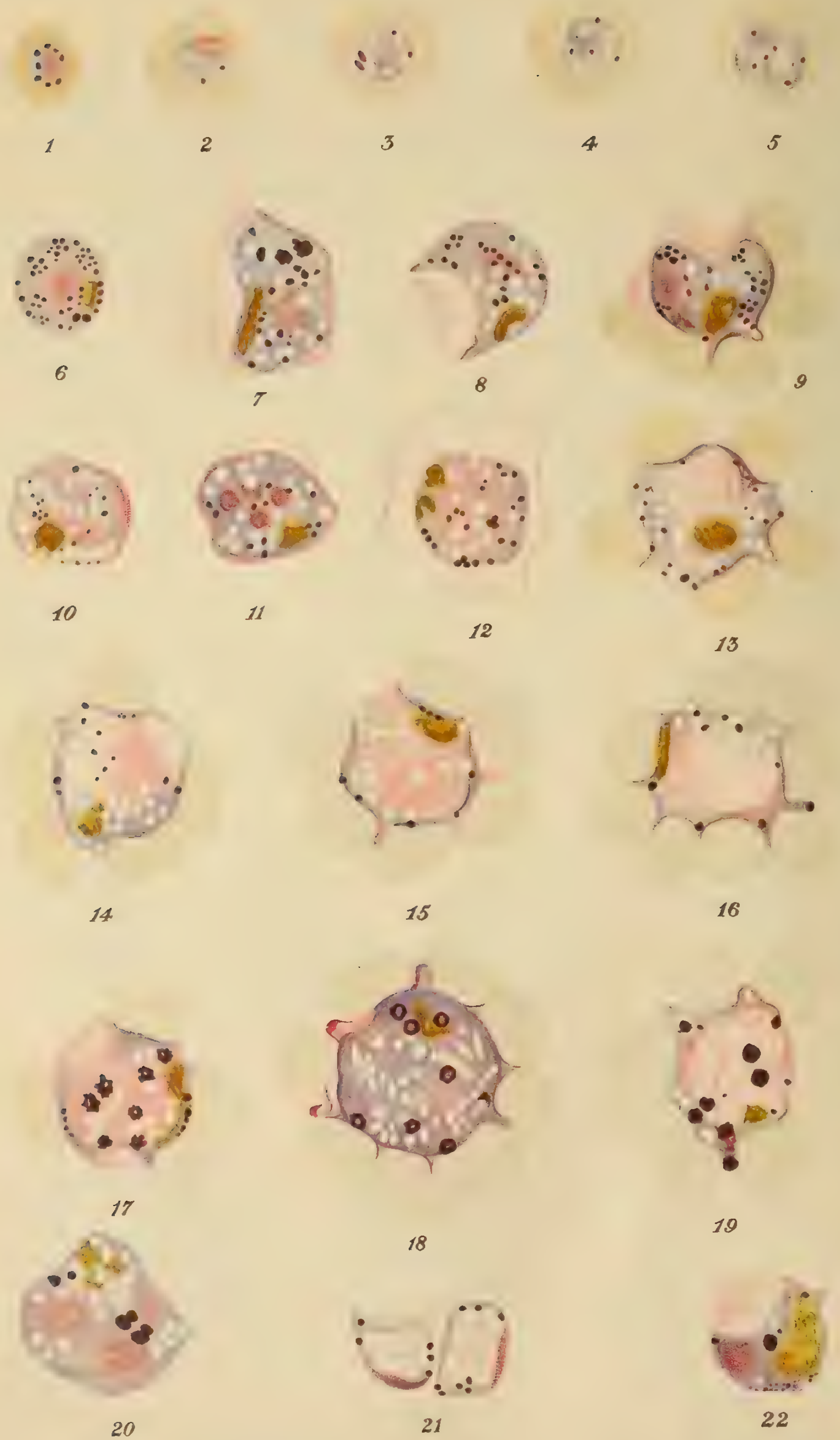
Bruce & others

Roy. Soc. Proc. B. vol. 87. Pl. 5.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Plasmodium cephalophi sp. nov.

M. E. Bruce, del.

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the other antelope was caught on the plateau and had been in captivity and not in the vicinity of other wild game for at least six months before it was brought to Kasu. As it developed an acute attack a few weeks after it had been placed in the same enclosure with the infected duiker, it would appear that in its case the infection was contracted locally.

The two antelope were first found to be infected in the height of the dry season (October), and although a systematic search was made for adult or larval mosquitoes, none could be trapped or found in or near the enclosure.

In the same compound there were also another young duiker, a young reedbuck, and a young hartebeeste, and although the blood of these animals was examined frequently, no malarial parasites could be found.

If this parasite should prove to be a new one, the name of *Plasmodium cephalophi* is proposed for it.

DESCRIPTION OF PLATES.

Plate 4.

Fig. 1.—Schizont, ruptured in making the film. The merozoites are drawn along in the direction of the spreading of the blood. The residual mass and free pigment are also shown.

Figs. 2 and 3.—Merozoites entering red cell. Note the granules in fig. 2 scattered throughout the protoplasm; in fig. 3 there are two granules, one large and one small.

Figs. 4–15.—Trophozoites, showing marked enlargement and paleness of red cells and concentration of portion of red cell in vacuole.

Figs. 16–18.—Young forms of "rosette" formation.

Fig. 19.—Fully developed schizont before cleavage of protoplasm.

Figs. 20–22.—Schizonts which show commencing cleavage of protoplasm, with a single mass of gamboge-coloured pigment.

Fig. 23.—Schizont in which the merozoites are completely separated. Some are oval and some circular in shape. All show a V-shaped group of granules at the opposite pole from the nucleus.

Stained Giemsa, × 2000.

Plate 5.

Figs. 1–5.—Young parasites, with faintly staining protoplasm and without vacuole. These were found in the same films as the forms shown in Plate 1, figs. 6–14.

Figs. 6–11.—Gametocytes, with faintly staining nucleus and granular protoplasm.

Figs. 12–16.—Forms found in the blood some weeks after acute attack (macrogametocytes).

Figs. 17–22.—Some anomalous forms.

Stained Giemsa, ×2000.

Trypanosomes of the Domestic Animals in Nyasaland. I. *Trypanosoma simiæ*, sp. nov. Part II.—*The Susceptibility of Various Animals to T. simiæ.*

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Introduction.

In a previous paper* the morphology of this interesting species of trypanosome was described, and it is now proposed to give an account of its action on animals.

One of the first interesting points to be noted about this species is that, as far as is known, the warthog (*Phacochœrus ætheopicus*) is the only animal among the wild game of this district which harbours it.† It is probable that it will also be found in the blood of the bush-pig, but not a single specimen of this animal has as yet been obtained by the Commission. The warthog is numerous in the low country in this neighbourhood, which accounts for the large number of tsetse flies found to be infected with *Trypanosoma simiæ*.‡

It is to be regretted that this species was not named after the warthog instead of the monkey, but at the time the name *Simiæ* was taken the Commission was ignorant of the close connection which exists between the former animal and this parasite.

Another interesting feature in regard to this trypanosome is the virulence it displays towards monkeys and the domestic pig, killing these animals in an incredibly short period of time, whereas it is harmless to oxen, antelope, dogs, and the smaller experimental animals. Curiously enough, this trypanosome also infects goats and sheep, although oxen and antelope escape.

The rapidity with which the virulence of *T. simiæ* becomes modified is also remarkable. When a cage containing wild *Glossina morsitans* is placed on a monkey and a goat, both animals take the disease, and the monkey in such an acute form that the average duration of life is only a few days. But if it is attempted to pass *T. simiæ* from an infected goat to a healthy monkey by the inoculation of the goat's blood, the experiment usually fails, showing that a short sojourn in the blood of the goat has almost nullified the virulence of the parasite for the monkey.

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* 'Roy. Soc. Proc.,' 1912, B, vol. 85, pp. 477–481.

† *Ibid.*, "Trypanosomes found in Blood of Wild Animals."

‡ *Ibid.*, "Infectivity of *Glossina morsitans* in Nyasaland."

T. simiæ belongs to the same group as *T. pecorum*, and it is curious that in the latter species this loss of virulence also occurs. If *T. pecorum*, which is usually more or less infective to the monkey, dog, and rat, lives for some time in the blood of the goat, it loses its power of infecting the other animals. This has given rise to the erroneous idea that a separate species—*T. nanum*—exists. *T. nanum* is in truth nothing but a strain of *T. pecorum* which has lost its virulence for these other animals by its passage through the goat.

T. simiæ is also like *T. pecorum* in general appearance, and in fact it is often difficult or impossible to distinguish between a short individual of the former species and a long one of the latter. The average length of *T. simiæ* is 17·5 microns, with a minimum of 14; the average length of *T. pecorum* is 14 microns, with a maximum of 18. When the coloured plates of the two species are compared this resemblance is at once seen. There is the same well-developed undulating membrane, the same oval nucleus, situated about the middle of the body, and the same eccentrically-placed micronucleus, often appearing to project beyond the margin, as noted in the description of the morphology of *T. simiæ*.

These two species, however, differ essentially in their action on animals. Whereas *T. simiæ* expends its virulence on monkeys and pigs, *T. pecorum* is especially fatal to cattle, goats, sheep, and dogs.

ANIMALS SUSCEPTIBLE TO TRYPANOSOMA SIMIÆ, SP. NOV.

Table I.

Date.	No. of expt.	Source of virus.	Period of incubation in days.	Duration of disease, in days.*	Remarks.
			Cattle.		
1912. April 24...	485	From Monkey 405......	—	—	Never showed trypanosomes.
„ 24...	486	„ 405......	—	—	„ „
July 17...	904	From Goat 653	—	—	„ „
„ 17...	945	„ 653	—	—	„ „
			Antelope.		
Sept. 25...	1240	From Monkeys 1258 and 1259	—	—	Duiker. Never showed trypanosomes.
Nov. 5...	1238	From Monkey 1541 ...	—	—	Reedbuck. Never showed trypanosomes.
„ 5...	1240A	„ 1541 ...	—	—	Duiker. Never showed trypanosomes.
1913. Mar. 5...	1980	„ 1910 ...	—	—	Reedbuck. Never showed trypanosomes.
„ 5...	1981	„ 1910 ...	—	—	Duiker. Never showed trypanosomes.

* Duration includes the days of incubation; it dates from day of infection.

Table I—*continued*.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Goats.		
1912.					
Feb. 1...	109	Wild flies	17	55	Mixed infection.
„ 1...	117	From Monkey 20	11	50	„ „
„ 3...	125	Wild flies	7	36	„ „
„ 9...	175	„	20	88	„ „
„ 19...	247	„	21	30	„ „
„ 27...	262	„	9	—	Still alive after 398 days.
Mar. 23...	363	„	6	42	Mixed infection.
April 19...	428	„	6	13	Died of *T. simiæ*.
„ 24...	425	From Monkey 405......	22	—	Still alive after 341 days.
„ 24...	426	„ 405......	—	—	Never showed trypanosomes.
„ 29...	429	Wild flies	12	42	Mixed infection.
May 14...	279	„	16	54	Died of *T. simiæ*.
„ 15...	416	„	5	19	Mixed infection.
„ 18...	553	„	11	112	„ „
June 1...	571	„	11	23	„ „
„ 4...	620	„	3	30	Died of *T. simiæ*.
„ 19...	653	From Goat 620	8	29	„ „
„ 19...	654	„ 620	8	107	„ „
„ 19...	710	„ 620	8	50	Mixed infection.
Aug. 21...	1117	From Warthog 1139...	40	—	Still alive after 222 days.
„ 24...	1113	„ 1186...	23	—	„ „ 219 „
Sept. 12...	1311	„ 1308...	18	—	Killed October 3.
„ 20...	1461	From Goat 1113.........	9	—	Still alive after 183 days.
Oct. 3...	1470	„ 1311.........	21	—	„ „ 179 „
„ 14...	1483	„ 1461.........	14	—	„ „ 168 „
Nov. 5...	1550	From Monkey 1541 ...	6	—	„ „ 146 „
„ 5...	1551	„ 1541 ...	6	—	„ „ 146 „
„ 5...	1552	„ 1541 ...	6	—	„ „ 146 „
„ 15...	1601	From Pig 1585	3	—	„ „ 136 „
„ 20...	1613	Wild flies	9	—	„ „ 131 „
1913.					
Jan. 27...	1810	„	6	52	Mixed infection.
			Sheep.		
1912.					
July 11...	907	From Goat 653	5	36	Mixed infection.
			Pigs.		
Nov. 5...	1553	From Monkey 1541 ...	6	6	Died of *T. simiæ*.
„ 5...	1554	„ 1541 ...	6	6	„ „
„ 5...	1555	„ 1541 ...	6	7	„ „
„ 11...	1585	From Pig 1555	4	5	„ „
„ 15...	1600	„ 1585	3	4	„ „
„ 18...	1609	„ 1600	3	4	„ „
„ 18...	1611	Wild flies	3	7	„ „
„ 23...	1631	From Goats 1113, 1434, 1461, 1470, and 1483	—	—	Never showed trypanosomes.
„ 25...	1636	Wild flies	13	98	Mixed infection.
Dec. 3...	1665	„	6	17	„ „
„ 6...	1674	„	3	10	„ „
„ 11...	1683	„	3	5	Died of *T. simiæ*.
„ 16...	1701	„	6	12	Mixed infection.
1913.					
Jan. 24...	1801	„	3	4	Died of *T. simiæ*.

* Duration includes the days of incubation; it dates from day of infection.

Table I—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
		Baboons.			
1912.					
April 30	499	From Monkey 449	—	—	Never showed trypanosomes.
,, 30	500	,, 449	—	—	,, ,,
,, 30	501	,, 449	—	—	,, ,,
		Monkeys.			
Jan. 20	20	Wild flies	7	9	Died of *T. simiæ*.
,, 24	55	,,	9	9	,, ,,
,, 29	54	,,	5	12	,, ,,
Feb. 2	49	,,	6	8	,, ,,
,, 6	59	From Monkey 55	6	8	,, ,,
,, 8	58	Wild flies	11	—	Still alive after 318 days.
,, 13	219	,,	7	9	Mixed infection.
,, 27	286	From Dog 211	6	52	,, ,,
Mar. 9	326	From Goat 175	—	—	Never showed trypanosomes.
Apr. 15	405	Wild flies	5	—	Killed April 24.
,, 18	447	,,	9	—	Still alive after 159 days.
,, 18	448	,,	8	12	Died of *T. simiæ*.
,, 19	404	,,	5	6	,, ,,
,, 19	449	From Monkey 404	3	10	,, ,,
,, 23	465	Wild flies	4	8	,, ,,
,, 24	480	From Monkey 405	8	10	,, ,,
,, 26	488	Wild flies	10	13	,, ,,
,, 27	492	From Dog 436	5	63	Mixed infection.
,, 27	495	Wild flies	7	11	Died of *T. simiæ*.
May 3	504	,,	12	11	,, ,,
,, 8	523	,,	4	5	,, ,,
,, 9	521	,,	5	7	,, ,,
,, 14	545	,,	3	6	,, ,,
,, 24	576	From Monkey 545	10	14	,, ,,
,, 31	601	Wild flies	6	31	Mixed infection.
June 11	629	,,	11	17	Died of *T. simiæ*.
July 17	906	From Goat 653	8	11	,, ,,
Sept. 18	1404	,, 1113	—	—	Never showed trypanosomes.
,, 18	1405	,, 1113	—	—	,, ,,
,, 28	1404	,, 1113	—	—	,, ,,
,, 28	1405	,, 1113	—	—	,, ,,
Oct. 3	1468	,, 1311	—	—	,, ,,
,, 3	1476	,, 1117	—	—	,, ,,
,, 14	1404	,, 1461	—	—	,, ,,
,, 14	1405	,, 1461	—	—	,, ,,
,, 23	1515	Wild flies	6	8	Died of *T. simiæ*.
,, 29	1404	From Goat 1483	—	—	Never showed trypanosomes.
,, 29	1405	,, 1483	—	—	,, ,,
,, 29	1535	,, 1483	—	—	,, ,,
Nov. 1	1541	From Monkey 1515	3	—	Killed for inoculation purposes.
,, 1	1542	,, 1515	3	11	Died of *T. simiæ*.
,, 11	1583	From Pig 1555	3	20	,, ,,
,, 11	1584	,, 1555	3	—	Still alive after 140 days.
,, 11	1586	Wild flies	7	12	Died of *T. simiæ*.
,, 19	1614	From Goat 1601	—	—	Never showed trypanosomes.
,, 19	1617	,, 1550	—	—	,, ,,
,, 27	1663	,, 1601	8	—	Still alive after 124 days.

* Duration includes the days of incubation; it dates from day of infection.

Table I—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
		Monkeys—*continued.*			
1912.					
Nov. 28...	1404	From Monkey 1586 ...	—	—	Never showed trypanosomes.
Dec. 7...	1677	From Goat 1550.........	—	—	,, ,,
,, 11...	1681	Transmission expt. ...	11	23	Died of *T. simiæ.*
,, 14...	1697	From Goat 1551.........	—	—	Never showed trypanosomes.
,, 14...	1698	,, 1601.........	—	—	,, ,,
1913.					
Jan. 16...	1772	Wild flies	14	—	Still alive after 74 days.
		Average	7·6	10·8	
		Dogs.			
1912.					
Feb. 6...	119	From Monkey 55	—	—	Never showed trypanosomes.
,, 6...	128	,, 55	—	—	,, ,,
,, 6...	130	,, 55	—	—	,, ,,
,, 16...	211	Wild flies	—	—	,, ,,
,, 24...	250	From Monkey 219......	—	—	,, ,,
,, 24...	251	,, 58......	—	—	,, ,,
,, 24...	254	,, 58......	—	—	,, ,,
,, 24...	258	,, 219......	—	—	,, ,,
Mar. 9...	320	From Goat 175	—	—	,, ,,
,, 9...	321	,, 125	—	—	,, ,,
,, 9...	319	,, 175	—	—	,, ,,
,, 9...	322	,, 125	—	—	,, ,,
,, 9...	325	From Monkey 286......	—	—	,, ,,
,, 13...	330	,, 58......	—	—	,, ,,
April 5...	409	,, 58......	—	—	,, ,,
,, 12...	436	Wild flies	—	—	,, ,,
,, 24...	481	From Monkey 405......	—	—	,, ,,
,, 24...	482	,, 405......	—	—	,, ,,
,, 27...	489	,, 449......	—	—	,, ,,
,, 27...	490	,, 449......	—	—	,, ,,
Oct. 3...	1469	From Goat 1311.........	—	—	,, ,,
,, 29...	1520	,, 1483.........	—	—	,, ,,
		Rabbits.			
Oct. 29...	1522	From Goat 1483.........	—	—	Never showed trypanosomes.
,, 29...	1523	,, 1483.........	—	—	,, ,,
Nov. 5...	1543	From Monkey 1541 ...	—	—	,, ,,
,, 5...	1544	,, 1541 ...	—	—	,, ,,
,, 5...	1545	,, 1541 ...	—	—	,, ,,
Dec. 14...	1543	From Pig 1636	—	—	,, ,,
,, 14...	1544	,, 1636	—	—	,, ,,
,, 14...	1545	,, 1636	—	—	,, ,,
,, 24...	1714	Wild flies	7	11	Mixed infection.
1913.					
Jan. 28...	1827	,, ...	9	—	Mixed infection. Still alive after 62 days.

* Duration includes the days of incubation; it dates from day of infection.

Table I—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
		Guinea-pigs.			
1912.					
April 24...	483	From Monkey 405......	—	—	Never showed trypanosomes.
Oct. 29...	1524	From Goat 1483.........	—	—	,, ,,
Nov. 5...	1546	From Monkey 1541 ...	—	—	,, ,,
,, 5...	1547	,, 1541 ...	—	—	,, ,,
1913.					
Jan. 4...	1731	Transmission expt. ...	—	—	,, ,,
		Rats.			
1912.					
April 24...	484	From Monkey 405......	—	—	Never showed trypanosomes.
,, 27...	491	,, 449......	—	—	,, ,,
Oct. 29...	1521	From Goat 1483.........	—	—	,, ,,
Nov. 5...	1548	From Monkey 1541 ...	—	—	,, ,,
,, 5...	1549	,, 1541 ...	—	—	,, ,,

* Duration includes the days of incubation; it dates from day of infection.

Action of T. simiæ *on Horses, Oxen, and Antelope.*—There has been no opportunity of testing the action of *T. simiæ* on equines. Four oxen were inoculated, two from an infected monkey and two from a goat, but all four remained in good health. Five antelope in confinement were also inoculated without result, and it would seem that these animals are really refractory, since in no instance has *T. simiæ* ever been found in the blood of antelope.

Disease set up in Goats and Sheep by T. simiæ.—Thirty-one goats and one sheep were infected by this parasite, as the result of various experiments. Fifteen of these were used in wild *G. morsitans* feeding experiments, three had warthog blood injected into them, and 14 were inoculated with the blood of infected experimental animals. It has already been remarked that this species of trypanosome varies rapidly in its virulence or power of setting up disease in animals. Its virulence would appear to be exalted by passage through the "fly," or, at least, to have reached its highest virulence after passage through the "fly," and lowered by passage through certain animals. For example, of the 15 goats infected by the bite of the "fly," 13 died, on an average, in 46 days, and only two recovered. The three goats which were infected by the direct injection of warthog blood all recovered; this is curious when it is remembered that in all probability the "fly" must get its infection solely from this animal. Six goats were injected with the blood of "fly"-infected monkeys; only one died, four recovered, and one proved refractory.

Six goats were also infected from goats; of these, three died and three recovered. This variation in virulence may also be expressed, for the sake of clearness, in the following table:—

Table II.—Mortality among Goats infected in Various Ways by *T. simiæ*.

	Mode of Infection.			
	Wild *G. morsitans* feeding.	Warthog blood, injection of.	Monkey blood, injection of.	Goat blood, injection of.
Percentage of deaths	86·7	0·0	16·7	50·0
No. of goats employed	15	3	6	6

What does this table show? It is meant to show that if goats are infected with *T. simiæ* directly by the bite of the "fly," most of them will die, whereas if the parasite is passed for a generation or two through goats, monkeys, or the warthog, the rate of mortality for the goat will fall. But it may be objected that the six goats which were infected with goat's blood have a mortality of 50 per cent., which is almost as large as the wild *G. morsitans* feeding experiments. But if Table I be referred to, it will be found that the three fatal cases were inoculated with blood from a goat which had only recently—some 10 days—been infected by the bite of the "fly," and ought, on account of the shortness of the time, to be included in the first, or "fly"-feeding, column; the other three, non-fatal cases, from goats infected by the injection of warthog's blood, should be included in the second column. If this were done, then the table would appear as follows:—

Table III.

	Mode of infection.		
	Wild *G. morsitans* feeding.	Warthog blood, injection of.	Monkey blood, injection of.
Percentage of deaths	90·0	0·0	16·7
No. of goats employed	18	6	6

It must be confessed, however, that the numbers are small and subject to a large margin of error, but the figures seem sufficiently remarkable to merit record. It may also be remarked that the matter is of little practical importance, as under natural conditions goats will always be infected by the bite of the "fly"; but it is interesting as showing the fallacy of judging from the action of laboratory strains as to what will occur in nature.

Among the goats infected by wild *G. morsitans*, there are many cases of mixed infection. On referring to Table I it will be seen that there are 15 cases of infection by wild *G. morsitans* among the goats. Of these, only three were cases of pure infection with *T. simiæ*, the remaining 12 were mixed infections. These three died, on an average, in 32 days. There were 12 cases of mixed infection; of these, one recovered and the remaining 11 died, on an average, in 49 days.

From this it would appear that *T. simiæ*, acting alone, is as rapidly fatal to goats as it is when occurring in various combinations with *T. brucei*, *T. pecorum*, or *T. capræ*. *T. simiæ* infection, when the result of the bite of the "fly," must therefore be looked upon as a fairly deadly enemy to goats.

During life this disease of goats is not marked by any special symptoms. There are neither swellings of the body, limbs, or face, nor corneal opacities, as is sometimes the case in other trypanosome diseases of goats: the animal merely becomes more and more anæmic and emaciated, and finally dies of exhaustion.

Disease set up in the Domestic Pig by T. simiæ.—In the whole range of the trypanosome diseases of animals there is surely nothing so striking as the rapidly fatal action of *T. simiæ* on the domestic pig. In nine experiments the average duration was only 5·3 days. This, not from the time of the appearance of the trypanosomes in the blood, but from the date of infection. Further, this rapid action is not the result of an exaltation of virulence by numerous passages through the pig, but natural to the trypanosome.

In regard to the symptoms of the disease during life, nothing noteworthy happens owing to the rapidity of the disease.

Disease set up in the Monkey by T. simiæ —This trypanosome is also remarkably fatal to the monkey. In 20 experiments with wild *G. morsitans* 17 monkeys died, the duration, on an average, being only 9·5 days from the day the flies were fed. Three are still alive after 318, 159, and 74 days, and have evidently recovered. When a monkey is inoculated with blood from an infected monkey the result is the same, the duration in five cases being 10·6 days. It will be seen, however, from Table I, that these five monkeys were all inoculated with blood from monkeys which had only very recently been infected by the bite of the "fly." On the other hand, when a monkey is inoculated with blood from a goat infected with *T. simiæ*, the result, as a rule, is negative. Ten monkeys were inoculated with blood of "fly"-bitten goats and only one became infected; the other nine remained negative although the trypanosome had only been in the goats an average of 23 days. From this it would appear that *T. simiæ* loses its virulence for the monkey if exposed to the action of the living goat's blood for even so short a period as 12 or 14 days.

Action of T. simiæ *on the Dog.*—The dog appears to be immune to this species of trypanosome. Many experiments were made by feeding wild *G. morsitans* on dogs and by inoculating the blood of infected goats and monkeys. In not a single case did the trypanosomes appear in the blood nor did the dogs appear to be affected in any way. But in two "fly"-feeding experiments (211 and 436), although *T. simiæ* did not appear in the blood of the dogs on microscopical examination, yet the injection of their blood into monkeys gave rise to an infection with *T. simiæ*, showing that the parasite was present although in numbers too small to be detected by the microscope. It is possible, then, that the dog may act as a reservoir of this disease, but most improbable that it does so to any practical extent.

Action of T. simiæ *on Rabbits.*—The rabbit seems also to be practically immune to this disease. The injection of blood from infected goats, pigs, and monkeys has no effect. But on two occasions after feeding wild *G. morsitans* on rabbits, *T. simiæ* appeared in their blood, in one case (Experiment 1714) in large numbers, in the other (Experiment 1827) only rarely. Both these were examples of mixed infection, the former of *T. simiæ* and *T. pecorum*, the latter of *T. simiæ*, *T. pecorum*, and *T. brucei*. It was attempted to infect a rabbit by feeding on it a fly which was known to be infective with pure *T. simiæ*, but with no result.

This susceptibility, or non-susceptibility, of rabbits to *T. simiæ* is perhaps not a very important matter, but the experiments go to show that the natural mode of infection of trypanosome diseases by means of the "fly" is probably the most effective.

Action of T. simiæ *on Guinea-pigs and Rats.*—Both these species of animals appear to be refractory.

Table IV.—The Average Duration of Life in Various Animals infected by *T. simiæ*, Nyasaland. Mixed infections are not included. The duration includes the days of incubation; it dates from the date of infection. The letter R stands for refractory.

	Ox.	Antelope.	Goat and sheep.	Pig.	Baboon.	Monkey.	Dog.	Rabbit.	Guinea-pig.	Rat.
Average duration in days	R	R	46·6	5·3	R	10·8	R	R	R	R
No. of animals employed	4	5	5	9	3	24	21	10	5	5

Table V.—The Percentages of Recoveries in Various Animals from *T. simiæ* infection. This table includes mixed infections.

	Ox.	Antelope.	Goat and sheep.	Pig.	Baboon.	Monkey.	Dog.	Rabbit.	Guinea-pig.	Rat.
Percentages ...	R	R	37·5	0·0	R	14·3	R	R	R	R
No. of animals employed	4	5	32	13	3	35	21	10	5	5

THE CARRIER OF T. SIMIÆ, NYASALAND.

In Nyasaland the carrier of *T. simiæ* is *G. morsitans*, of which 3·4 per 1000 were found to be infected. A paper on the development of *T. simiæ* in *G. morsitans* is in course of preparation.

THE HOST, OR RESERVOIR, OF T. SIMIÆ.

The warthog.—Thirty-three of these animals were examined, and *T. simiæ* found in three.

CONCLUSIONS.

1. *T. simiæ* belongs to the same group as *T. pecorum*, and, like the latter is erratic in its action on animals.
2. *T. simiæ* affects goats, sheep, pigs, and monkeys. Oxen, antelope, dogs, rabbits, guinea-pigs, and rats are practically immune.
3. The carrier is *G. morsitans*.
4. The reservoir of the virus is the warthog.

Trypanosome Diseases of Domestic Animals in Nyasaland.
I.—Trypanosoma simiæ, *sp. nov.* *Part III.*

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[PLATES 6–8.]

INTRODUCTION.

In previous papers* the morphology of *Trypanosoma simiæ* and its action on animals have been described. In this it is intended to give an account of its development in *Glossina morsitans.*

Before entering, however, into the details of this particular development, it may be of interest to take a general survey of the various modes of development which take place in the different groups of trypanosomes. It may then be laid down that there is a well marked separate and characteristic mode of development in each of the three main groups of trypanosomes:—

In the first group—the *T. brucei* group—which includes *T. brucei*, *T. gambiense*, *T. evansi* (?), and *T. equiperdum* (?), the parasites develop—at least in the first two named species—at first through the whole length of the intestinal tract, excluding the proboscis, and eventually reach the salivary glands, where forms resembling those found in the blood of animals are developed, and these alone constitute the infective stage.

In the second group—the *T. pecorum* group—which includes *T. pecorum* and *T. simiæ*, the development takes place in the intestinal tract, including the labial cavity of the proboscis, and afterwards the trypanosomes reach the hypopharynx, or termination of the salivary duct in the proboscis. Here they revert to the original blood form and become infective. In this group trypanosomes are never found in the salivary glands, and no blood forms or infective forms are developed until the hypopharynx is reached.

In the third group—the *T. vivax* group—which includes *T. vivax*, *T. uniforme*, and *T. capræ*, the initial stages of the development take place in the labial cavity of the proboscis alone; later the hypopharynx is invaded, where again blood forms are developed, which again constitute the only infective forms. Here also there is no invasion of the salivary glands, and, in addition, no development takes place in the intestinal canal.

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* 'Roy. Soc. Proc.,' 1912, B, vol. 85, and 1913, B, vol. 86.

In all three groups the common factor which leads to the formation or development of the final or infective forms is the invasion of the salivary tract, and this is accompanied by a reversion to the original blood forms.

DEFINITIONS.

In this paper the word "proboscis" will mean the piercing apparatus of the "fly," made up of the labrum, labium, labellum, and hypopharynx. There are two tubes in the proboscis: one for the passage inwards of blood, made up by the coalition of the labrum and labium, the other for the passage outwards of the salivary secretion—the terminal salivary duct or hypopharynx. The term "labial cavity" in this paper will mean the former, or tube for conveyance of blood, the word "hypopharynx" the latter, or duct for conveyance of saliva. In the past the use of the word "proboscis," including both tubes, has given rise to a good deal of ambiguity.

The definition of the words "infected" and "infective" were given in a previous paper.*

The term "blood form" means a stage in the development of the trypanosomes in the "fly," when there is a reversion to the original form found in the blood of animals, and from which the cycle of development originated.

THE DEVELOPMENT OF T. SIMIÆ IN G. MORSITANS.

Eight experiments were carried out with laboratory-bred flies. Two were positive and six were negative. The following table shows these eight experiments, the number of flies used, the number of infected flies found on dissection and the number of days which elapsed before the flies became infective:—

Table I.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.
1912.					
May 1	502	20	−		
June 27	754	31	+	2	50
Oct. 9	1477	17	−	1	
Nov. 11	1582	8	−		
„ 15	1602	20	−		
„ 21	1622	16	−		
1913.					
Feb. 5	1847	45	+	7	20?
„ 10	1856	16	−		

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* 'Roy. Soc. Proc.,' B, vol. 86. ("Infectivity of *Glossina morsitans* in Nyasaland.")

It will be noted that there is a great difference between the two positive experiments as regards the time required for the flies to become infective. In the first 50 days elapsed, in the second only 20. This is due to different temperatures under which the experiments were carried out. The first positive experiment was done during the coldest time of the year on Kasu Hill, when the mean temperature was 62° F. (16·6° C.), which is much lower than on the plains, the natural habitat of *G. morsitans.* The flies in the other experiment were kept in an incubator at a temperature of 83° F. (28·3° C.), and they became infective much sooner.

Details of the Two Positive Experiments.

The following table gives the principal details of the first positive experiment:—

Experiment 754.

Table II.

Day of expt.	Procedure.	Remarks.
1–3	Flies fed on *T. simiæ*-infected monkey.	Trypanosomes first appeared on the 57th day.
4	Starved.	
5–60	Fed on clean monkey.	

It is seen that it was not until the flies had been fed on the clean monkey for 57 days that the animal showed trypanosomes in its blood. If we allow seven days for the average incubation period of the parasite in the mammalian host, then the monkey contracted the disease about the 50th day after the infecting fly had fed on trypanosome-infected blood.

The following table gives the principal details of the second positive experiment:—

Experiment 1847.

Table III.

Day of expt.	Procedure.	Remarks.
1–10	Flies fed on *T. simiæ*-infected monkey.	Flies became infective on the 26th day after first infected feed; 16 days after the last.
11	Starved.	
12–27	Fed on clean monkey.	

Since the flies of this experiment were fed on infected blood for a period of 10 days, the time required for the trypanosomes taken up by the "fly" to multiply and regain their virulence cannot be accurately estimated. Allowing seven days for the incubation period it cannot be more than 20 days.

When the healthy monkey became infected, in order to separate the infective flies, those remaining alive were divided into three batches. Each batch was put into a cage and fed separately on a healthy monkey. The following table gives the details and results of feeding the three batches of flies:—

Table IV.

Expt.	Batch.	No. of flies.	No. of days fed.	Result.	No. of infected flies found.
1847	1	12	7	–	0
1847	2	10	7	+	3
1847	3	13	7	+	3

The monkeys on which Batches 2 and 3 were fed showed trypanosomes in their blood on the sixth day after the first application of the flies. It is therefore highly probable that the flies infected the monkeys on the first day of feeding.

Details of the Six Negative Experiments.

The following table shows the method of procedure in carrying out the six negative experiments:—

Table V.

Expt.	Day of expt.	Procedure.	Remarks.
502	1–2	Fed on infected monkey.	
	3–4	Starved.	
	5–42	Fed on clean monkey.	All flies negative on dissection.
1477	1–3	Fed on infected goat.	One infected fly found on the 40th day; proboscis and gut infected.
	4–5	Starved.	
	6–45	Fed on clean monkey.	
1582	1st	Fed on infected pig.	
	2nd	Starved.	
	3–30	Fed on clean monkey.	All flies negative on dissection.
1602	1st	Fed on infected pig.	
	2nd	Starved.	
	3–35	Fed on clean monkey.	All flies negative on dissection.
1622	1st	Fed on infected pig.	
	2nd	Starved.	
	3–29	Fed on clean monkey.	All flies negative on dissection.
1856	1–7	Fed on infected monkey.	
	8–26	Fed on clean monkey.	All flies negative on dissection.

In Experiment 1477 a portion of the intestine of the infected fly was inoculated subcutaneously into a pig; the pig did not become infected.

Out of a total of 173 flies used in these experiments, 10 flies (5·8 per cent.) became infected with a growth of trypanosomes in the intestines and in the probosces. It will also be seen that only 1 fly in 31 (2·7 per cent.) became infective when the flies were kept at ordinary room temperature, whereas 4 became infected in 45 (9 per cent.) when the flies were kept at a temperature of 28° C.

GENERAL CONSIDERATIONS REGARDING THE DEVELOPMENT OF T. SIMIÆ IN G. MORSITANS.

All the flies dying during the progress of the experiments were dissected. In the two positive experiments, out of 76 flies dissected, nine infected flies were found. The following table gives the results of the dissection of these nine flies :—

Table VI.

Expt.	Time, days.	Proboscis.		Proventriculus.	Crop.	Fore-gut.	Mid-gut.	Hind-gut.	Proctodæum.	Salivary glands.
		Labial cavity.	Hypopharynx.							
754	37	+ +		+ +	–	+ +	+ +	–	–	–
754	50	+ +		+ +	–	+ +	+ +	–	–	–
1847	16–26	–	–	–	–	+ +	+ +	–	–	–
1847	30–40	+	+	–	–	+ +	+ +	–	–	–
1847	31–41	+ +	+	+ +	–	+ +	+ +	+ +	–	–
1847	31–41	+ +	–	+	–	+ +	+ +	+ +	–	–
1847	31–41	+ +	+	+		+ +	+ +			
1847	32–42	+ +	+	+	–	+ +	+ +	+ +	–	–
1847	32–42	–	–	+	–	+ +	+ +	–	–	–

From this table it will be seen that in seven out of nine flies dissected the labial cavity is found to contain trypanosomes. This is very different from what is seen in the similar table relating to *T. gambiense*. There not a single case of infection of the proboscis is recorded.*

At what stage in the development of the trypanosome the proboscis takes a part is not known. It is probable that the infection commences in the intestinal tract and moves forward into the proboscis, but owing to the difficulty of obtaining sufficient laboratory-bred *G. morsitans* the Commission have not, up to the present, enough evidence to establish this detail.

In the two infected flies found in the cage of flies, Experiment 754, it is to be regretted that the contents of the hypopharynx were not noted, but in all the infected flies found in Experiment 1847 this was done, with the result that the hypopharynx was found invaded by trypanosomes in four out of the seven.

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* 'Roy. Soc. Proc.,' 1911, B, vol. 83, p. 516.

Plate 6 represents, at a magnification of 500 diameters, the labial cavity and hypopharynx of an infected fly. While the labial cavity contains clusters of large ribbon-like trypanosomes, the hypopharynx is swarming with small active forms resembling the original blood forms, from which the developmental cycle arose. When the plate is examined the facility with which a tsetse fly can infect an animal will no longer be a matter of wonder.

Finally, from the table it will be seen that in no case were the salivary glands invaded.

THE METHODS USED IN THE EXAMINATION OF THE FLIES.

The flies were dissected as described in a previous paper.* An additional method of examining the contents of the hypopharynx was to isolate infective flies by putting each fly into a separate tube, numbering it, and feeding the fly on a susceptible animal with a corresponding number on its cage. The numbers on the cages of animals which became infected indicated the tubes containing infective flies. These, when thus identified, were starved for 24 hours, in order to make them hungry. A tube containing one of the infective flies was then taken, and its mouth being covered with mosquito netting was applied to a large cover-glass placed on a man's finger. The hungry fly at once attempted to feed through the glass, and in poking about with its proboscis smeared the surface of the cover-glass with saliva. This was immediately fixed, stained with Giemsa, and examined.

THE TRYPANOSOMES FOUND IN THE PROBOSCIS.

Reference to the table above will show that in Experiment 754 two infected flies were found, one on the 37th day after feeding on an infected monkey, and one on the 50th day, and that the labial cavities of both flies were infected.

The fly that died on the 50th day was the one which no doubt actually infected the healthy monkey, since the animal showed trypanosomes seven days after the death of this fly and no other infected fly was found. As these two flies died before they were isolated, the method of inducing them to salivate on a cover-glass was not used. When, however, the two proboscides were examined in a drop of normal saline solution under a cover-glass, trypanosomes attached to the labrum were seen growing in colonies in the labial cavity. They were moving freely and some detached individuals were swimming actively up and down the lumen of the tube.

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* 'Roy. Soc. Proc.,' 1911, B, vol. 83, p. 513.

It is to be regretted that the contents of the hypopharynx were not specially noted. These were two of the earliest experiments, and at that time the contents of the labial cavity and the hypopharynx were not differentiated.

In Experiment 1847 seven infected flies were found. It will observed (Table VI) that the first was dissected on the 16th day after the last infected feed and that the proboscis was not infected. Another fly dissected on the 32nd day had also no infection of the proboscis. A third fly dissected on the 31st day had the labial cavity of the proboscis infected but not the hypopharynx. The remaining four were found to contain swarms of trypanosomes in both the labial cavity and the hypopharynx. On examination it was observed that there were two distinct varieties. One found in the hypopharynx closely resembled small blood forms of *T. simiæ* (Plate 8, figs. 18 to 21). They swarmed in the narrow tube, which had the appearance of being blocked up by their enormous numbers. These small blood forms of the parasite were readily distinguishable from those growing in the labial cavity. Those growing in the labial cavity resemble *Leptomonas*, and are peculiar in having their non-flagellated extremity prolonged to a snout-like extension (Plate 8, figs. 12 to 16). They are assembled in clusters and attached by their flagella to the inner surface of the labrum, their prolonged free extremity moving vigorously in the lumen of the tube (Plate 6, fig 1).

The contents of the hypopharynx of a living infective fly isolated from Batch 2 was examined by inducing the fly to salivate on a cover-glass as described above. On examination of the stained preparations typical blood forms of *T. simiæ* were seen embedded in the saliva which the fly had ejected on the cover-glass in its efforts to reach the skin (Plate 8, figs. 22 to 25). Another infective fly was taken alive from its glass tube and its proboscis gently squeezed until a minute drop of fluid was observed at its tip, which was then lightly rubbed over a cover-glass. Here again typical blood forms of *T. simiæ* were found embedded in the salivary secretion (Plate 8, figs. 26 to 29).

In Experiment 1847 it is seen that a positive result is associated with the finding of infected flies in which *T. simiæ* resembling those found in the blood of infected animals are found in large numbers blocking up the tube of the hypopharynx.

The experiment of tempting the infective fly to feed through a cover-glass demonstrates the fact that when the fly salivates, as it undoubtedly does in puncturing the skin, these blood forms of the parasite are washed out of the hypopharynx with the saliva and are injected with it under the skin of the fly's victim.

Further examination of flies by inducing them to salivate on cover-glasses revealed the fact that sometimes the long, narrow intestinal forms of trypanosomes are ejected in large numbers on to the cover-glass (Plate 8, figs. 1 to 11). There is no doubt, therefore, that an infected fly has the power of regurgitating the contents of its proventriculus and intestines forward into the labial cavity and probably into the blood stream of the bitten animal. It is conceivable that in this way the proboscis first becomes infected by the intestinal forms of trypanosomes, which attach themselves to the inner surface of the labrum and enter the lumen of the hypopharynx, which they invade, however, only as far as the entrance of the two salivary ducts. Here in the chitinous hypopharynx they establish themselves and, bathed in the salivary secretion, finally complete their development into the infective blood form of the parasite.

It is a curious fact that neither the salivary glands nor even the salivary ducts beyond the hypopharynx have ever been found infected with *T. simiæ*.

It was proved by the Commission in Uganda that the blood forms of *T. gambiense* developing in the salivary tract were the virulent forms of the parasite, and it now seems also proved that the developmental forms of *T. simiæ* found in the hypopharynx represent the last and infective stage of development of this species of trypanosome in the "fly."

It may be noted here that in the negative Experiment 1477, in which an infected fly was found (Table V), the labial cavity was infected with the long forms of the parasite attached to the labrum, but most careful search failed to reveal infection of the hypopharynx with blood forms. In this fly the parasite had not attained the final and essential stage of its development—the reversion to the blood type—and so the fly was harmless.

THE TRYPANOSOMES FOUND IN THE ALIMENTARY CANAL.

The intestines of infected flies were generally packed full of trypanosomes from the proventriculus to the mid-gut. Sometimes the infection extended to the hind-gut, but never beyond.

Little need be said in regard to the developmental forms found in the intestines. One curious fact, however, emerges and that is, that it is impossible to differentiate one species of trypanosome from another by the study of these intestinal forms. Whether it is *T. brucei* or *T. gambiense*, *T. pecorum* or *T. simiæ*, they present the same appearance. Perhaps on further work some differences may become apparent, but at present no difference has been found to exist. The most numerous forms are long, slender, ribbon-like, very active trypanosomes, which in a former paper* were called the normal reproductive type. By comparing Plate 7 with the insets of that paper, the resemblance between the intestinal developmental forms of *T. simiæ* and *T. gambiense* will be apparent.

CONCLUSIONS.

1. That *T. simiæ* can be transmitted from infected to healthy animals by the tsetse fly *G. morsitans.*

2. That *T. simiæ* multiplies in the intestines and in the labial cavity of the proboscis of the "fly." Here only developmental forms are found, never infective forms.

3. That the *T. simiæ* growing in the intestines of the "fly" has no specific characters by which it can be distinguished from other species of pathogenic trypanosomes found in tsetse flies.

4. That the final stage of the development takes place in the hypopharynx, wherein the infective form of the parasite, similar in shape to the trypanosome found in the blood of infected animals, is produced.

5. That the flies do not become infective until about 20 days after their first infected feed.

DESCRIPTION OF PLATES.

PLATE 6.

Fig. 1.—Appearance of the labial cavity of the proboscis of *Glossina morsitans* with *Trypanosoma simiæ* growing in clusters attached by their flagellar extremities to the inner surface of the labrum. Living and unstained, × 500.

Fig. 2.—Appearance of the hypopharynx in the same fly, showing innumerable small and active *T. simiæ* almost blocking up the lumen of the duct. Living and unstained, × 500.

PLATE 7.

Intestinal developmental forms of *T. simiæ.* These do not differ in appearance from the developmental forms of other species of pathogenic trypanosomes found in the intestinal tract of tsetse flies.

PLATE 8.

Developmental forms of *T. simiæ* from the labial cavity and hypopharynx of *G. morsitans.*

Figs. 1–11.—Trypanosomes ejected by a living *G. morsitans* on attempting to feed through a cover-glass. These are supposed to be intestinal forms pressed up into the proboscis and on to the glass by the muscular contraction of the fly.

Figs. 12–16.—*T. simiæ* growing in the labial cavity of the proboscis of *G. morsitans.*

Fig. 17.—Aberrant form from hypopharynx.

Figs. 18–29.—Blood forms of *T. simiæ* found in the hypopharynx. These form the final stage in the developmental cycle of this species of trypanosome and are the only infective forms. Stained Giemsa, × 2000.

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* 'Roy. Soc. Proc.,' 1911, B, vol. 83, p. 513.

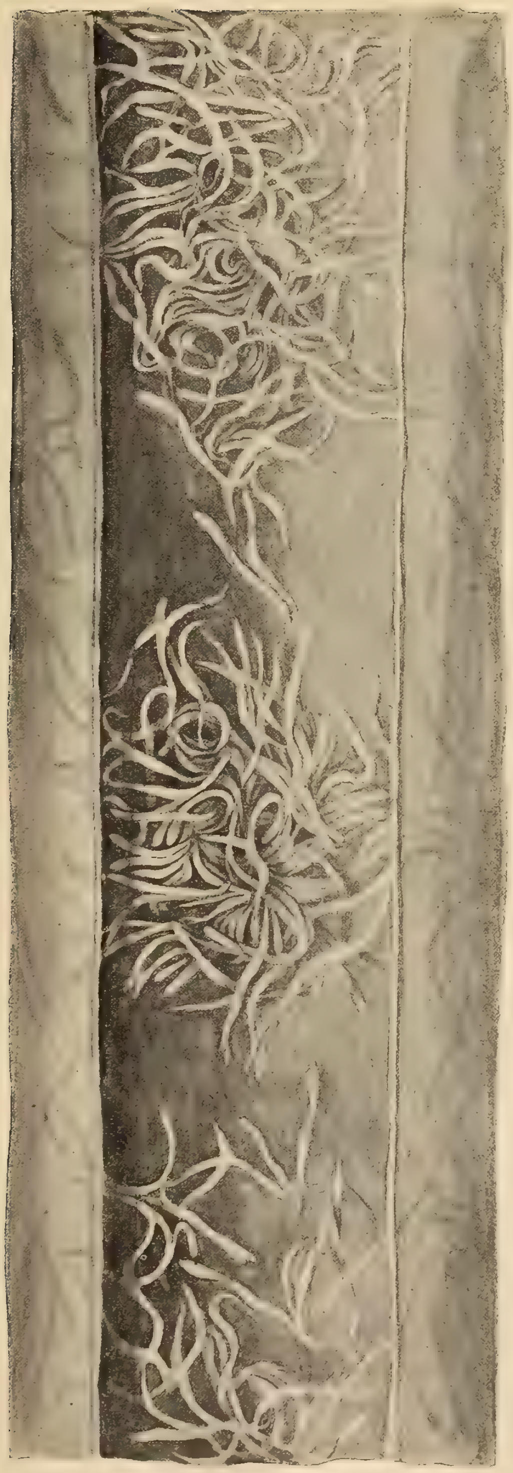


FIG. 1.

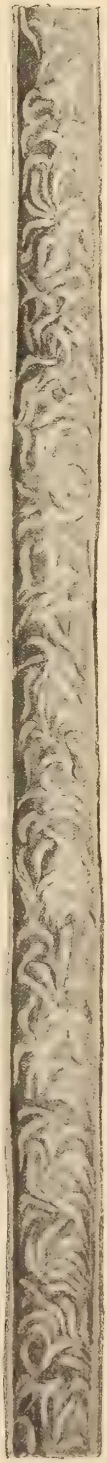
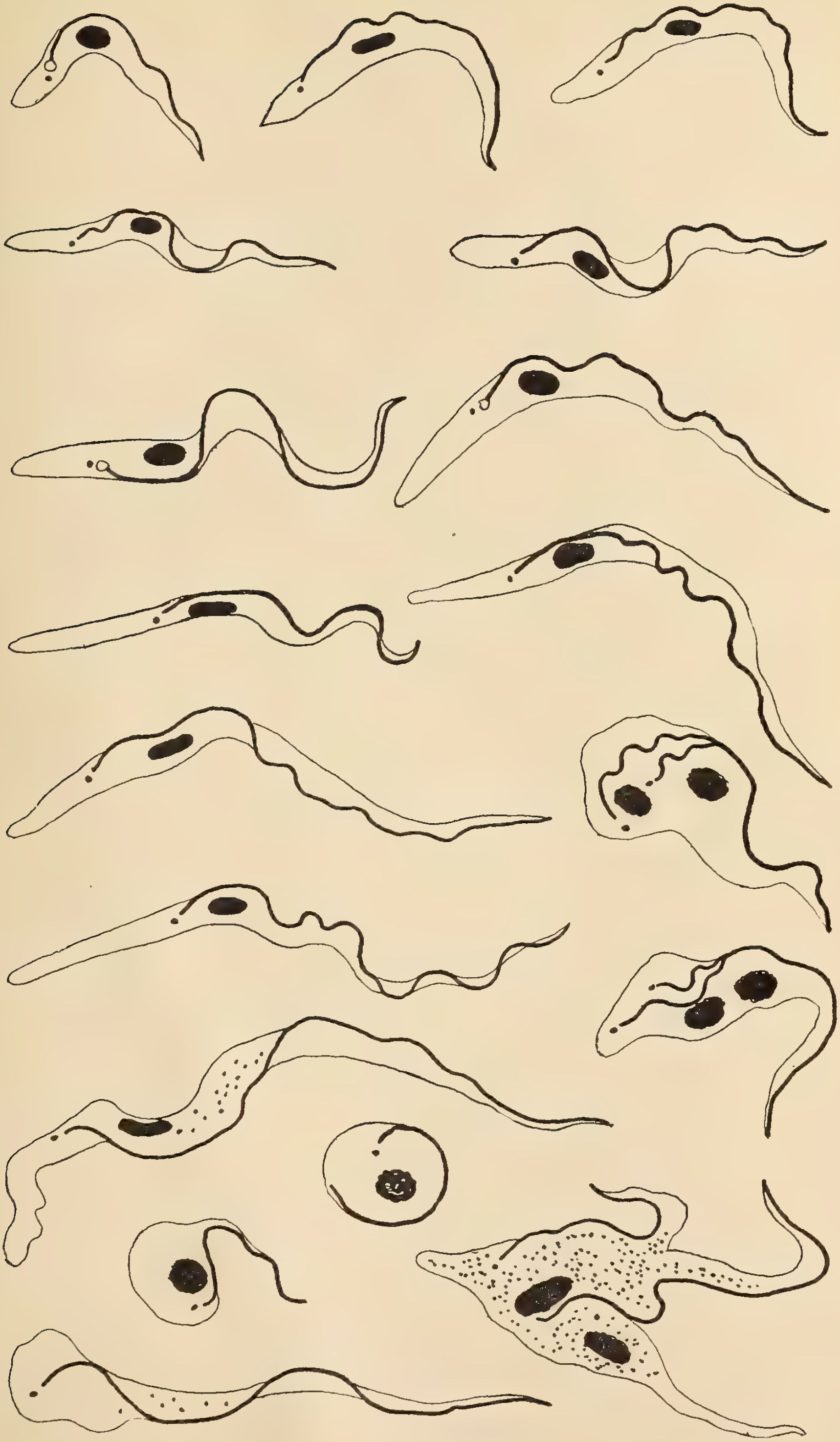


FIG. 2.

T. simiæ in labrum and hypopharynx.

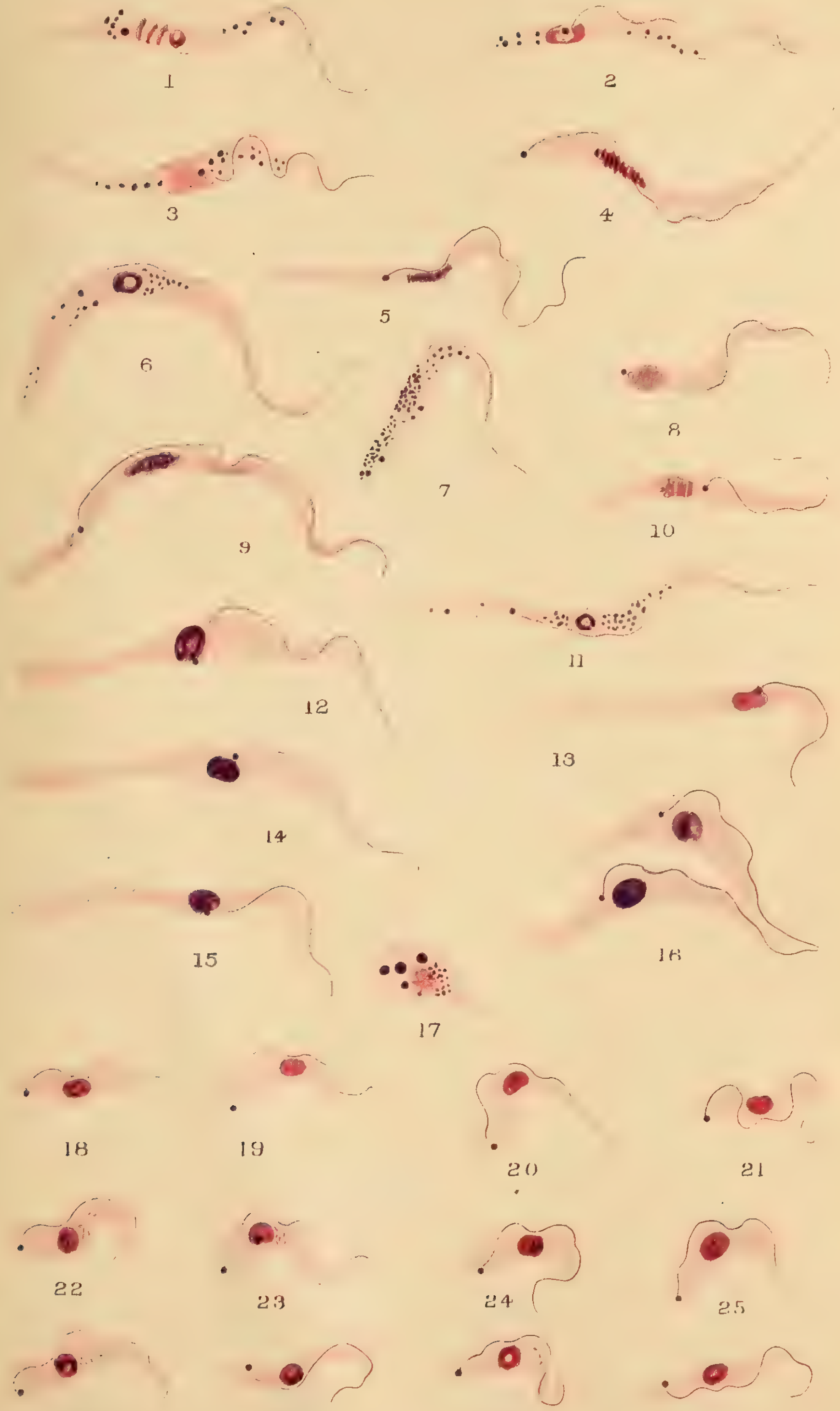
M. E. Bruce, del.

×750.



T. Simiæ in gut of fly.

Major Hamerton, del *x 2000.*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M F Bruce del. × 2000 Huth lith et imp.

T. simiæ from proboscis & hypopharynx.

Notes on Toxoplasma gondii.

By HELEN L. M. PIXELL, B.Sc., Beit Memorial Research Fellow.

(Communicated by Prof. E. A. Minchin, F.R.S. Received April 12,—
Read May 29, 1913.)

[PLATE 9.]

The genus *Toxoplasma* was founded by Nicolle and Manceaux (3, 4, 5) for *T. gondii*, a species discovered by them parasitic in the gondi (*Ctenodactylus gondii*) in Tunisia. When first discovered the parasite was identified as a new species of *Leishmania*. Further investigation showed, however, that its resemblance to the true *Leishmania* type was quite superficial, since it neither possessed a kinetonucleus nor gave rise to flagellated forms in cultures, consequently it was made the type of the new genus *Toxoplasma*, of which the affinities and systematic position are at present very doubtful. Since then other species of *Toxoplasma* have been described from rabbits in Brazil (*T. cuniculi*, Carini), from dogs in Italy and Brazil (*T. canis*, Mello), from moles in Japan (*T. talpæ*, Prowazek), and from pigeons in Brazil (*T. columbæ*, Yakimoff and Kohl-Yakimoff [10]).

Very recently Nicolle and Conor (6) have given an account of the parasitism of *T. gondii* and the reactions of susceptible experimental hosts.

In January of this year Dr. Nicolle most courteously presented Prof. Minchin with some mice, two of which had been inoculated from an infected pigeon at Tunis. These were brought to the Lister Institute and handed over to me for study. I am indebted to Prof. Minchin not only for the privilege of examining so interesting a parasite but also for much kind help and advice.

The two infected mice died shortly after their arrival in England: one, in fact, on the night after it arrived in London. In order to preserve the strain of the parasites, other mice or pigeons were inoculated by Prof. Minchin from those that died. I have thus been able to make *post-mortem* examinations of animals that had died recently from the effects of the parasite or had been freshly killed.

Although I propose in this memoir to deal chiefly with the minute structure and reproduction of *Toxoplasma*, which has never yet been studied by cytological methods, I may also refer briefly to some points in the occurrence of the parasites and their effects on the experimental hosts that have come under my observation in the course of the work.

Course of the Infection in Mice and the Distribution of the Parasite in the Tissues.

Mice are very susceptible to the effects of the parasites, which prove fatal in from 5 to 15 days after intraperitoneal inoculation. The symptoms are: (1) an increased rate of respiration until the last 12 hours or so, when breathing becomes slow and laboured; (2) a general loss of the senses, especially sight—the mice appearing to be blind for the last day or two; (3) general lethargy—food, however, is taken in many cases almost as usual until nearly the end.

The individuals showed many *post-mortem* differences, which will be considered under the different tissues.

(1) *Peritoneal Fluid.*—In some cases the peritoneal cavity is full of a viscid, slightly cloudy fluid containing numerous parasites, both free and intracellular—in Mice C and D (see below) this was the case and these two were undoubtedly the best infected specimens—Mouse F, however, which had an equally large amount of fluid, seemed to have nothing like the number of parasites, not more, in fact, than A, in which there was hardly any peritoneal fluid. It will be noticed, too, that in both A and F the course of infection lasted seven days.

Other anomalous cases will be noticed among the results of experiments tabulated below, from which it would seem that the quantity of peritoneal fluid cannot always be correlated with the number of parasites present, nor is the rapidity with which they prove lethal necessarily proportional to their number. On the other hand D had, perhaps, the best infection, and it was the most rapidly fatal, whereas B, in which the disease lasted 12 days, was found on *post-mortem* examination to have only a very poor infection.

The parasites in well infected animals are to be found in numbers both free in the fluid and enclosed in the cells floating in it. Of the leucocytes they are nearly always mononuclears (Plate 9, fig. 1) that are affected; only occasionally has a polymorphonuclear been found to contain one or two parasites. In other cases the parasites are embedded in, or attached to, cellular debris (figs. 2, *a–c*); the origin of these masses of debris is sometimes difficult to determine—they are referred to by Nicolle (5, p. 98) as "gangues." Still more numerous, however, are the parasites in endothelial cells (macrophages) which have evidently become detached from the peritoneum and float freely in the fluid (figs. 3 and 4).

(2) *Mesenteries.*—Finding that detached endothelial cells were so often infected with parasites, I was led to examine the mesenteries themselves, with the result that the endothelial cells forming these serous membranes were found in many cases to be packed with parasites (figs. 5 and 6).

Major S. R. Christophers tells me that the serous membranes have not, so far as he is aware, been examined in cases of Kala Azar, and he suggests that the systemic parasite, *Leishmania donovani*, may possibly be found to infect the membranes and peritoneal fluid in patients suffering from this disease also, since the two parasites seem to be similar in so many other ways.

In preparations of the stretched omentum, stained with silver nitrate and the other reagents mentioned below, large pavement cells may be seen crowded with as many as sixty or seventy parasites (fig. 6), other cells may have only one or a few toxoplasms (fig. 5). In the latter case the nucleus of the host-cell sometimes retains its normal oval contour with one or two distinct nucleoli (fig. 5, *a*), but in all those enclosing more than two or three parasites the nucleus has become more or less rounded, with the chromatin in several blotches, giving a decidedly necrotic appearance. In fig. 5 it will be seen that the central cell (*a*) is uninfected and normal; the right-hand one (*b*) contains two parasites and already the nucleolus is beginning to break up. In the left-hand cell (*c*) the degeneration of the nucleus has advanced farther still, though not so far as the nuclei of the cells represented in figs. 4 and 6. In these, as in many other cases, two such necrotic nuclei are present, which fact seems to suggest that after nuclear division had taken place, the infected cell had not sufficient vitality for the division of the cytoplasm.

The long, narrow, endothelial cells, which in many cases show branching ends, are also very often full of parasites, and although the nucleus in these may remain more or less oval, it could not be mistaken for that of a normal cell, owing to its general necrotic appearance. In transverse sections of the mesenteries parasites could be distinguished not only in the flat endothelial pavement cells forming the serous membrane, but also in the connective tissue corpuscles of the subserous areolar tissue. None, however, was observed free in the lymphatics or capillary blood-vessels, nor in their endothelial linings.

In mice, such as B, I, and J, described below, which were found with only very few parasites in the peritoneal fluid, the mesenteries also seemed to be destitute of them, and in such cases very few toxoplasms could be found anywhere in the body.

(3) *Liver.*—The parasites here are less numerous than in the peritoneal fluid. When present they are frequently seen to be dividing, and may be free (figs. 13–15), or in the mononuclear leucocytes, never apparently in hepatic cells. In Mouse L, in which the infection lasted 15 days, the liver had become pale and friable, and was much hypertrophied.

(4) *Spleen.*—This organ generally has fewer parasites than the liver. They may be included in mononuclears or be free.

(5) *Blood.*—The peripheral blood does not appear to contain parasites. It has been tested in some cases during life.

The heart-blood was found to contain a few parasites in the two cases examined.

(6) *Kidney.*—No parasites were found in this organ in the one case examined.

(7) *Lungs* and pleural fluid contain a few toxoplasms, at any rate in some cases.

(8) *Aqueous Humour and Cornea.*—No parasites could be found in the two cases examined (Mice C and J).

(9) *Brain.*—No parasites were found in Mouse J.

(10) *Bone Marrow.*—No parasites were found in Mouse L.

(1) *Experimental Infection of Mice.**

Mice A and B were inoculated from the liver of a slightly infected pigeon which was killed at Tunis on January 10. In all cases inoculation was intraperitoneal from peritoneal fluid unless otherwise stated.

Mouse A.—Died January 17. Course of infection seven days.

Only a little peritoneal fluid was present, but a number of parasites were found, both free and intracellular. The liver contained a few, chiefly intramononuclear parasites.

Mouse B.—Died January 22. Course of infection 12 days.

Very little peritoneal fluid and only a few parasites. Liver and spleen scarcely any parasites.

Mouse C.—Inoculated January 17 from A. Died January 25. Course of infection eight days.

Large amount of peritoneal fluid and many parasites here and in mesenteries. Left eye opaque, but no parasites found in aqueous humour or cornea, and lens appeared to be normal.

Mouse D.—Inoculated January 25 from C. Died January 30. Course of infection five days.

Large amount of peritoneal fluid and numerous parasites in this and the mesentery.

Mouse E.—Inoculated January 30 from D. Died February 5. Course of infection six days.

Not much peritoneal fluid nor very many parasites.

Mouse F.—Inoculated February 5 from E. Died February 12. Course of infection seven days.

Large amount of peritoneal fluid, which was, however, poor in parasites.

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* These inoculations were performed by Prof. Minchin under his licence.

Mouse H.—Inoculated February 12 from F. Died February 20. Course of infection eight days.

Very little peritoneal fluid, and this was poor in parasites, so also the mesentery. Liver, spleen and heart-blood contained a fair number.

Mouse I (with three legs only).—Inoculated February 20 from H. Found dying January 26 and chloroformed. Course of infection six days.

Very little peritoneal fluid and a very poor infection. Lungs and liver found to be infected with a bacillus and also a diplococcus. The presence of the latter would suggest that the mouse was dying of pneumonia. Inoculation of peritoneal fluid into another mouse gave no result.

Mouse J.—Inoculated February 20 from the liver of H. Died March 3. Course of infection 11 days.

Only a little peritoneal fluid with a few parasites. The mesentery also only contained a few. In the pleural fluid occasional specimens were found, as also in the lungs, heart-blood, and liver.

Mouse L.—Inoculated March 3 from J. Died March 18. Course of infection 15 days.

A quantity of fat present and the animal seemed generally well nourished. A fair amount of peritoneal fluid, but it only contained a few parasites. Liver hypertrophied and of a somewhat friable consistency, but it only contained a small number of parasites and none could be found in the bone marrow.

Mouse G was fed February 5, 12, and 20 on material infected with parasites, but was apparently none the worse on March 19.

Conclusions—

(1) Infection would not seem to take place in nature by means of the alimentary canal unless the parasite may possibly be swallowed in a form different from that in the vertebrate host.

(2) The disease ran its longest course in Mice B, J, and L, which were found in *post-mortem* examination to have only a few parasites. If the rapidity with which the poorly infected Mouse I succumbed be ascribed in part to its pulmonary bacterial infection and general unhealthy condition, then it would seem that the length of the course of infection is approximately inversely proportional to the number of parasites in the whole body and that the death of the host may be due to their cumulative toxic action.

(2) *Pigeons.*

Pigeon 1.—Inoculated January 22. Intraperitoneum from peritoneal fluid of Mouse B. No effect.

Pigeon 2.—Inoculated January 30. Intraperitoneum from peritoneal fluid of Mouse D. February 26, peripheral blood tested, no parasites found.

March 19, appeared in perfectly normal health.

I cannot account for this pigeon not becoming infected after being inoculated with the peritoneal fluid of Mouse D, which contained numerous parasites.

[*June* 19.—I understand from Dr. Nicolle that he has also found that pigeons cannot be infected by inoculation of the virus after passing through mice, though he has succeeded in infecting them easily directly from gondi.]

Technique.

Many smears were stained with Giemsa, and the presence of parasites was easily tested in this way, although, as is well known, this stain cannot be relied upon for cytological detail. On the whole iron hæmatoxylin was found to be by far the best nuclear stain. Delafield's hæmatoxylin also gave good results. Twort's stain, borax carmine, paracarmine, Mayer's acid hæmalum and Mann's hæmatein were used with less success. Double staining was found to be advisable in all cases. Orange G and eosin were quite satisfactory for this purpose. No differential staining of the cytoplasm was effected by using mixtures such as licht-grün and picric acid or picronigrosin; both of these were, however, useful for the stretched omentum. Another useful mixture for sections was eosin and licht-grün made according to Chatton's formula (2, p. 254), which is a modification of Prénant's. The cytoplasm of the parasite took up the eosin only, so apparently there are no reserve food-particles or other green-staining inclusions.

When fixed by osmic acid vapour and absolute alcohol for Giemsa staining, the parasites, as usual, appear much larger (figs. 7 and 8) than when fixed by the wet methods generally employed. For the latter Maier's fluid gave very good results—slightly better perhaps than Flemming's fluid or a mixture of corrosive sublimate and acetic acid.

Morphology of the Parasite.

The living parasites are non-motile, but seem to be capable of slightly altering their shapes. A specimen drawn with a camera lucida at intervals of 5 or 10 minutes is shown in figs. 2, *a–e*. It appeared to be trying to free itself from a mass of cellular debris. I would not like to say for certain, however, that it was really changing its shape, for the apparent differences in appearance may possibly have been due to slight invisible currents in the medium causing the parasite to be viewed from different aspects. I have also observed them apparently bending in the middle and turning over.

In size, as can be seen from the figures, they agree very closely with the measurements given by Nicolle (5, p. 99) for the average parasite from the gondi, namely, 5–5·5μ in length by 3–4μ in breadth.

The nucleus is generally clearly visible as a rounded region, clearer than the rest of the body and somewhere near its centre (figs. 9 and 10). Fig. 11 shows a dividing form with two nuclei.

In some specimens definite round refringent granules are to be seen, sometimes only one as in fig. 9, but never in large numbers. These will be referred to again later.

In preparations stained by one of the exact cytological methods it can easily be seen that the nucleus is of the protokaryon type, that is, it consists of a sharply defined karyosome suspended in a clear vesicle (fig. 12). The nucleus is probably bounded by a membrane, which is, however, in no case distinct, and only occasionally is there any peripheral chromatin (fig. 13). The karyosome, no doubt, encloses a centriole, since a centrodesmose is formed during fission, as will be described later, but it has not been found possible to differentiate the staining of the small karyosome in order to make the centriole apparent.

In the alveolar cytoplasm there are often fairly large vacuoles, and the refringent granules seen in living specimens take up chromatin stains very readily, and are probably, I think, of the nature of reserve chromatin, or volutin, as described by Reichenow (6, pp. 328–331) for *Hæmogregarina stepanowi.* In some cases they have almost exactly the size of the karyosome, and since they may occur quite close to the nucleus (figs. 7, 14, 15, 17, and 22), they are apt to be somewhat confusing.

The parasites generally lie in distinct vacuoles in the protoplasm of the host-cell (figs. 3–5); the formation of these might possibly be attributed to the reaction on the part of the cell by which it throws out some sort of secretion round the parasite, as suggested by Row (7, p. 749) in the case of *Leishmania tropica,* but it seems more likely to indicate a destructive liquefying action by the parasite on the protoplasm of the host-cell. This latter explanation would account for the fact that cells infected with many parasites are in an advanced state of necrosis.

Free parasites may be of different sizes and are frequently found undergoing fission. Intracellular forms often appear to have divided repeatedly in rapid succession, giving rise to forms which are much reduced in size (fig. 4, *s*). Those nearer the boundary of the cell seem to break away at intervals as shown in this figure, and all that is seen to remain of some cells is a degenerating nucleus with a fringe of cytoplasm.

Multiplication of the Parasite.

Toxoplasma divides by the simplest form of binary fission. The karyosome elongates and becomes dumb-bell shaped (figs. 16 and 17). The two daughter-karyosomes then move apart from one another, remaining connected by a short centrodesmose (figs. 18 and 19). This soon appears to snap, for it has disappeared by the time the stages represented in figs. 20 and 21 are reached. The vesicle is then constricted off and the two daughter nuclei separate (figs. 22–25).

Division of the body is usually longitudinal, but may sometimes apparently be transverse, or oblique (fig. 24). Fig. 22 shows two daughter-individuals which have evidently just been formed by longitudinal division, and one appears to be again dividing longitudinally, the other transversely.

In some infected cells, perhaps owing to the fact that the parasites can divide in different directions, compact more or less spherical masses of 20 or more parasites may be produced which have something the appearance of a cyst. A spherical mass of this kind is seen to be forming at *s* in fig. 6 and probably also in figs. 4 and 5. The first of these is, however, scarcely half the size of the masses seen in many infected cells. Nicolle (5, p. 99) suggests that this appearance may have led Splendore (9) to interpret erroneously similar masses as cysts in the rabbit. In the schizogony, recently described by Yakimoff and Kohl-Yakimoff (10, p. 202) as taking place in free or intracellular forms, multiple fission into 32 or more may apparently take place, but it is impossible to make out the details of the process from their figures, made from Giemsa preparations.

Attempts to make Cultures of the Parasite.

Attempts were made to cultivate the parasite on agar plates, on blood agar, and in blood serum, also by adding to some peritoneal fluid a small percentage of 50-per-cent. dextrose as recommended by Bass (1) for the cultivation of the Malaria parasite, but in no case could any different form be produced.

This seems to be all that can be done here in connection with *Toxoplasma*, but I hope to carry on this work next month in the desert of South Tunisia, which is the most northerly haunt of its natural host—the gondi—and there to investigate its method of transmission and life-history.

[*June* 19.—In the above I have purposely refrained from discussing the affinities of *Toxoplasma* until such time as its life-history should be known, for until then its systematic position must remain uncertain. The parasite is, however, to all appearances a true Protozoon. Since Roche-Lima* has claimed to show that *Histoplasma* is a yeast-like organism, the question naturally arises as to the possibility of *Toxoplasma* being also related to the Blastomycetes. That such is not the case, however, is, I think, sufficiently proved by the characteristics described above, such, for example, as: (1) the constant appearance of the nucleus; (2) the absence of a distinct refringent membrane round the parasite; (3) the fact that it does not grow in cultures; and (4) that no sign of gemmation has been observed, the parasite generally dividing by longitudinal fission. Beer-wort gelatine was used for culture experiments in addition to the media mentioned above, but in no case could the *Toxoplasma* be made to grow, neither would they retain any colour when preparations were stained by Gram's method.

Nicolle and Conor (6) in their recent paper, which I saw for the first time in Tunis on my way to the desert, gave some evidence of the fact that *Toxoplasma* causes only a seasonal disease in the gondi. This I was able to confirm, for during April, when I was at Matmata (the place from which the disease was originally recorded by Dr. Nicolle), I could find no trace of *Toxoplasma*, although I examined 55 gondi and numerous other indigenous animals, including rats, mice, shrews, many birds, such as finches, pigeons and eagles, also a palm lizard, snakes, and frogs. I also carefully examined the ectoparasites of the gondi, these being in nearly every case the hexapod larvæ of a mite (*Trombidium* ?) clustered together, forming yellow masses in the ears, and ticks which Prof. Nuttall and Mr. Warburton have kindly identified for me as *Rhipicephalus* (*Pterygodes*) *fulvus*, Neumann. The latter were all in the nymph stage, but some have already metamorphosed since I brought them to England, and I am now hoping to make the adults feed on mice, guinea-pigs, or rabbits infected with *Toxoplasma*. Prof. Mesnil has most kindly sent me the virus for these experiments from Paris. In neither of the natural ectoparasites of the gondi could I discover anything that could be recognised as *Toxoplasma*, nor do I think that the parasite could be present in an unrecognisable form, for no results have been obtained by inoculating their contents into mice, gondi, and a pigeon. Dr. Nicolle most kindly performed these inoculations for me, and has kept the pigeon, gondi, and some of the mice under observation at the Pasteur Institute, Tunis; owing to the great delicacy of the gondi, it would not have been safe to risk bringing them to London.

I am very much indebted to Dr. Nicolle also for most kindly making excellent arrangements for my visit to the desert, and for providing me with plentiful reagents and apparatus for my work there. I should also like to thank the French military authorities for their courtesy and kindness during my stay at the Matmata Fort, where they gave me all possible assistance.]

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* 'Centr. für Bakter.,' 1913, Abt. 1, vol. 67, pp. 233–249.

EXPLANATION OF PLATE 9.

Unless otherwise stated, the preparations were stained with iron hæmatoxylin and counterstained with licht-grün and picric acid.

Fig. 1.—Mononuclear leucocyte enclosing several toxoplasms. × 2000.

Fig. 2.—Living specimen drawn at intervals of 5 or 10 minutes. × 2000. *a*, *b*, still attached to cellular debris; *c–e*, after becoming free.

Fig. 3.—Detached endothelial cell containing two parasites. × 1000.

Fig. 4.—Detached endothelial cell with two nuclei and several parasites, some of which are being set free. × 1000.

Fig. 5.—Three endothelial cells of the serous membrane. × 1000. *a*, normal cell; *b*, cell with two parasites and nucleolus already beginning to break up; *c*, cell with a further degenerated nucleus, and several rapidly dividing parasites forming a mass at *s*.

Fig. 6.—Cell from the serous membrane of the omentum with more than 60 parasites, some forming a compact mass at *s*. × 1000.

Fig. 7.—A toxoplasm from the liver of Mouse H, showing chromatoid masses in its cytoplasm. × 2000. Stained Giemsa.

Fig. 8.—Free dividing form from liver. × 3000. Stained Giemsa.

Figs. 9 and 10.—Living specimens from peritoneal fluid with one or more refringent granules in cytoplasm. × 2000.

Fig. 11.—Living specimen with two nuclei from peritoneal fluid. × 2000.

Fig. 12.—Intracellular parasites from a transverse section of omentum. × 3000. Stained iron hæmatoxylin, eosin, and licht-grün.

Fig. 13.—Form with some peripheral chromatin in the nucleus. × 3000. Stained iron hæmatoxylin and orange G.

Figs. 14 and 15.—Recently-divided forms with extranuclear chromatoid masses probably consisting of volutin. × 2000. 14. Stained iron hæmatoxylin and fuchsin S.

Figs. 16–25.—Free parasites showing binary fission. × 3000. 25. Stained iron hæmatoxylin and orange G.

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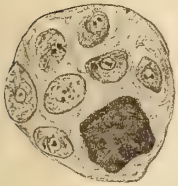

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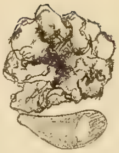

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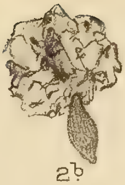

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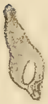

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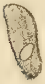

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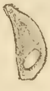

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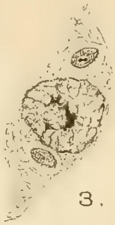

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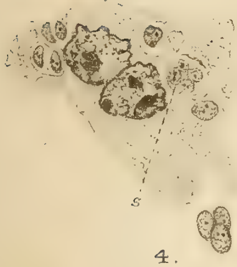

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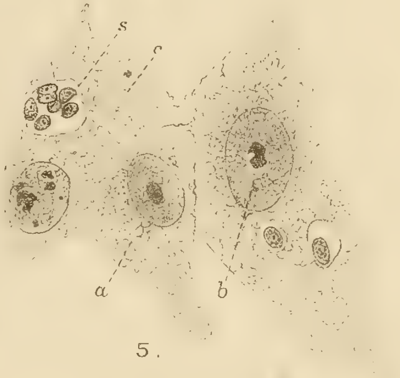

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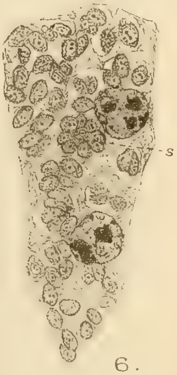

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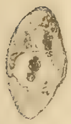

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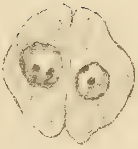

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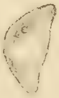

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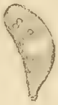

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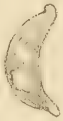

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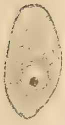

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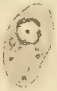

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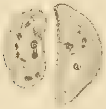

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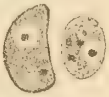

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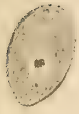

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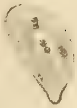

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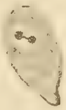

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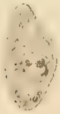

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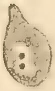

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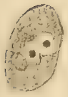

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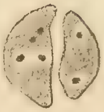

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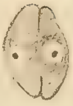

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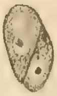

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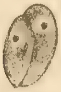

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The Growth and Sporulation of the Benign and Malignant Tertian Malarial Parasites in the Culture Tube and in the Human Host.

By John Gordon Thomson, M.A., M.B., Ch.B. Edin. (Pathologist to the Royal Southern Hospital, Liverpool, and Durning-Lawrence Research Student); and David Thomson, M.B., Ch.B. Edin., D.P.H. Cantab. (Clinical and Pathological Research Assistant, School of Tropical Medicine, Liverpool).

(Communicated by Sir Ronald Ross, K.C.B., F.R.S. Received May 21,—Read June 12, 1913.)

[Plate 10.]

Prefatory Note.

Researches on the cultivation of the parasites of malaria in Liverpool were commenced some time ago at my suggestion by Dr. Sinton, and then, with better success, by Drs. J. G. Thomson and McLellan, and by Dr. D. Thomson. We are greatly obliged to Sir Edwin Durning-Lawrence, Bart., for giving us the services of Dr. J. G. Thomson for this important enquiry.—Ronald Ross, 21st May, 1913.

Introduction.

The successful cultivation of malarial parasites was first announced by Bass and Johns (1912). Since then several workers, Thomson and McLellan (1912), Thomson, J. G., and Thomson, D. (1913), and Ziemann (1913), have successfully repeated these cultivation experiments. This achievement has led the way to new discoveries regarding the malarial parasite, and suggests that it may be possible to cultivate *in vitro* any protozoal parasite, however specialised it may be.

So far, only the asexual generation of the malaria parasite has been grown *in vitro.* The next step is to cultivate the sexual generation as it occurs in the human host and in the mosquito. We have attempted both, but, so far, without definite success. We have no doubt, however, that this will also be accomplished sooner or later. In 1912, Thomson, J. G., and Sinton successfully cultivated the human trypanosome, and the development they obtained was apparently that which takes place in the stomach of the tsetse fly. Joukoff (1913) states that he has cultivated the mosquito cycle of the malarial parasite, though his results have not yet been confirmed. There is no reason to doubt that the phases of development of protozoa in insects may be produced in the culture tube.

Cultivation Technique.

Our method of cultivation is practically the same as that of Bass and Johns, except that it is less complicated ; 10 c.c. of blood is drawn from a vein and transferred to a sterile test-tube containing a thick wire leading to the bottom of the tube from the cotton wool plug. 1/10 c.c. of a 50-per-cent. solution of glucose is added to this tube, preferably before adding the blood. The blood is defibrinated by gently stirring with the thick wire. Defibrination should be complete in about 5 minutes. The wire with the clot is then removed and the blood is poured into several smaller sterile tubes (about 1-inch column of blood in each). A rubber cap is placed over the cotton wool plugs to prevent evaporation and the tubes are then transferred (standing upright) to an incubator at a temperature of 37° to 41° C. The corpuscles settle in a short time, leaving about half an inch of clear serum at the top. It is apparently unnecessary to remove the leucocytes by centrifugalisation.

Further Observations on Cultivation.

We have grown four complete generations of parasites in one tube by the above method, and we do not see why their growth should not continue indefinitely, provided fresh serum and corpuscles be added. It is not the presence of leucocytes which prevents further development. This is due to degenerative changes taking place in the corpuscles and serum. If the serum and corpuscles be kept in a sterile condition in an ice chest they remain unchanged for a long time, but at the temperature required for the growth of the parasites visible changes take place in a few days. The corpuscles become fragmented and form a brownish debris and the serum becomes dark brown in colour. When this occurs the parasites are unable to continue their developmental cycle. Bass and Johns, in their original paper (1912), stated that the parasites grew only on the surface layer of corpuscles, and that no growth took place in the deeper layers. They also stated that the serum destroyed the parasites when they escaped from the corpuscles, so that when the spores escape they must enter immediately into a contiguous corpuscle in order to survive. The blood also, in their opinion, required to be heated to a temperature of 40° C. to destroy the complement in the serum; furthermore, in order to cultivate several generations, the leucocytes had to be removed. We do not believe that they have sufficient evidence for these deductions. We find that the parasites develop even in the very deepest layer of the column of corpuscles. Also it seems rather a contradiction that the parasites should grow best on the surface layer of corpuscles, next to the serum and the leucocytes which are supposed to destroy them. No doubt the leucocytes ingest some of the parasites, but they are never able to ingest all of them. Again, in one of our most successful cultures the temperature of the blood never exceeded 38° C., and we have found that they are able to grow at a temperature as low as 36° C. This is rather against the theory of complement destruction. In an able paper by Mary Rowley Lawson (1913), considerable evidence is brought forward to show that the parasites are extra-corpuscular during their entire development. If this is true, then the parasites would be constantly in contact with the serum which is supposed to destroy them. It appears to us that the only conditions necessary for the successful cultivation of the parasites are fresh corpuscles, fresh serum, a temperature of 37° to 41° C. and the requisite amount of glucose. The presence of leucocytes and complement is apparently immaterial.

The Optimum Temperature.—In our opinion the optimum temperature for cultivation is about 38° C. On two occasions we incubated identical culture tubes from the same patient, some at 37° C. and some at 41° C. On both the occasions the parasites developed much more successfully in the tubes incubated at 37° C.

Hæmolysis in the Culture Tubes.

This is a rare occurrence, having been seen only once out of 15 cases. A kind of hæmolysis occurs after the blood has remained in the incubator for several days. The corpuscles degenerate into a brownish debris, and the serum shows a brown discoloration.

Clumping of the Malignant Tertian Parasites.

This phenomenon has been observed in all our malignant tertian cultures (12 cases). It occurs even when the parasites are scarce. It is best observed in wet films, as smearing of the blood tends to break up the clumps. No tendency to clumping occurred in our benign tertian cultures.

Resistant Forms of Parasites.

Sometimes it will be found that the parasites do not grow well *in vitro*. They may only grow partially, stopping short of segmentation. This is liable to occur if quinine has been given to the patient before the blood is drawn. In other cases, only a few of the parasites reach maturity, these, apparently, being able to resist successfully adverse conditions in the culture tube.

The Morphology of Plasmodium falciparum *in Culture.*

The blood examined before incubation always showed the typical small ring parasites of malignant tertian malaria; these varied in size, with a maximum diameter of about 3 μ. There was no enlargement of the red blood corpuscles, and no Schüffner's dots. Fig. 1 (Plate 10) shows a small ring parasite from the peripheral blood before incubation. After many experiments in Liverpool, it has been found that there is great variation in the rate of growth of *P. falciparum* in artificial media, and many suggestions can be offered to explain this phenomenon. J. G. Thomson and S. W. McLellan (1912) found in one case that maximum sporulation of *P. falciparum* occurred in 25 hours. This culture was made from a case with very heavy infection of parasites, and the patient had taken no quinine. The temperature of the incubator was only 38° C., but the glucose added was slightly in excess of that recommended by Bass (1912). The rapid segmentation of the plasmodia in this case, therefore, may have been due to two causes: (1) the age of the parasites when introduced into the culture tube, since it is to be noted that they were fairly large rings about 3 μ in diameter, and (2) the quantity of glucose may in some way have hastened the growth. In other experiments, however, where the glucose was slightly in excess, there was no such rapid growth, so that in all probability the real cause of the rapid segmentation of the parasites *in vitro* is due to the age of the plasmodia when drawn for incubation purposes. Another factor which seems to influence the rate of growth is the previous administration of quinine, and this may, in fact, inhibit the growth entirely. On several occasions we have made unsuccessful attempts to cultivate the malignant tertian parasite, and these have usually been from patients to whom quinine had been administered.

On two occasions complete sporulation did not take place till after 50 hours' incubation at 41° C. On one of these occasions the parasites completed several generations, as illustrated by the accompanying coloured plate (figs. 1–20). We found that maximum segmentation took place in 52 hours. Division of the chromatin into daughter-cells began in about 36 hours (figs. 4 and 5). In 47 hours the number of spores had increased (figs. 6 and 7), and more or less complete segmentation took place in 52 hours. Fig. 11 shows a parasite which has produced 32 daughter-cells, and these have broken loose from the corpuscle. It is to be noticed that in the peripheral blood the small ring parasites show no pigment. After incubation, the rings gradually grow in size (fig. 3), and at a certain stage, immediately previous to the splitting of the chromatin, a round compact mass of pigment appears, usually situated near one margin of the parasite, which now measures about 5–6 μ in longest diameter. In the culture under discussion, this round mass of pigment was seen in 36 hours (figs. 4 and 5). J. G. Thomson and S. W. McLellan (1912) found that the pigment had collected in 12 hours. The parasites immediately previous to segmentation measure about 5–6 μ in their longest diameter, and all show the circular mass of compact pigment. We may call this stage the presegmenting stage. The chromatin now begins to split into two, and, if segmentation is completed, 32 daughter-cells may be formed (fig. 11). All stages of segmentation can be found from two spores up to 32 spores as a maximum. During segmentation, the circular mass of pigment takes a central position, and the spores form in a circular arrangement around these (figs. 7–11). In this culture segmenting forms were found at different stages up to 56 hours, this being no doubt due to the fact that the parasites were not all of the same age when introduced into the culture tubes. In 75 hours all segmenting forms had disappeared, and only very young parasites were found, about 1·5 μ in diameter and containing no vacuole (fig. 13). This represents the beginning of a second generation. It is to be concluded, therefore, that many of the young merozoites escape ingestion by the leucocytes, and enter a new red cell. The same culture examined in three days again showed segmenting forms (fig. 14), but in this case the spores were never so numerous as 32, and this can easily be explained by the adverse conditions which have now developed in the culture tube. These conditions did not, however, prevent the young parasites attempting to segment. In four and a half days (fig. 15) we again only found young rings, which represent the beginning of a third generation, and in six days sporulating forms were again found (fig. 16). Here only eight spores formed, and it was evident that the power of the parasite to undergo full segmentation was getting gradually less. On the seventh day, again, only very young plasmodia were found, which represented a fourth generation, and these again attempted to sporulate (figs. 18 and 19) on the eighth and ninth days respectively. On the tenth day only young parasites again were found, which represented a fifth generation. No further development, however, occurred, as the conditions in the culture were gradually becoming more and more adverse. We have thus evidence that the parasites will, under very suitable conditions, actually proceed in the original culture tube through four complete generations without the medium being in any way renewed.

We now wish to draw particular attention to several points of great interest which occur in the cultures of *P. falciparum*. In these there is a definite tendency for the parasites to clump together into masses immediately the circular mass of pigment appears, and even before segmentation begins. This tendency to clump becomes more marked when sporulation actually commences. In our opinion this phenomenon explains the reason why sporulation occurs as a rule in the capillaries of the internal organs. We seldom see sporulation or even presegmenting forms in the peripheral blood of malignant tertian cases, and the phenomenon of clumping explains this in a satisfactory manner. All the large parasites tend to get caught in the fine capillaries of the internal organs, and thus only young rings whose containing corpuscles have not yet acquired the clumping tendency are seen in the peripheral circulation. This fact explains many phenomena of pernicious malaria, and shows how comatose malaria may occur in infections with *P. falciparum*.

The Morphology of P. falciparum *in the Human Host.*

This is identical in every respect with that obtained *in vitro*, so that it is hardly necessary to describe it in detail. As already stated only the young ring forms of this parasite are found in the peripheral blood as a rule; this is due to the fact that when these parasites have grown larger than the ring stage, the containing corpuscles stick together, forming clumps which are unable to circulate, and thus are arrested in the fine capillaries of the inner organs. The study of the further stages of this parasite is obtained by examining smears of the inner organs such as the spleen, liver, brain, etc., of cases which have died. The autopsy smears of over one hundred cases have recently been examined by one of us (D. T.), in conjunction with Dr. W. M. James, in Panama. In such smears one finds the parasite in different stages of development. In some, presegmenters only are found; some show only young rings, and others only sporulating forms. This depends entirely on the stage of development of the parasite at the time of the patient's death. In some cases, of course, several stages of development are found, since one patient may contain several broods of parasites which are in different stages of development. In only one case did we find the parasites in the stage of maximum sporulation. All of them contained over 20 spores, and 13 per cent. had 32 spores. We think that there can be little doubt that we have proved conclusively, both from observations in the culture tube and in human host, that *P. falciparum* is capable of producing a maximum, under favourable circumstances, of 32 spores. This is a much larger number than has been previously given in the writings of competent observers. Ross (1912) quotes the figure of Welch (1897) at 6–20 or more spores. Later, however, from observations made by himself and D. Thomson, he taught that the maximum number was 2 to the power of 5 or 32, Marchiafava, Bignami, and Mannaberg (1894) state that the numbers vary from 8 to 15, while the following authors give the numbers as follows: Stephens and Christophers (1908), 8–10; Deaderick (1910), 5–25 and even 30; Gulland and Goodall (1912), 8–15. It is probable that these discrepancies are due to two reasons, (*a*) observations of autopsy smears in which the sporulation had not reached its full maturity, and (*b*) observations on autopsy smears of patients to whom quinine had been given before death. In such cases incomplete and atypical sporulation is seen. We do not claim, of course, that 32 spores are always produced even under favourable conditions without quinine administration. It is likely that the numbers produced vary considerably, just as in the case of benign tertian, which produces numbers varying from 16 to 26.

The Morphology of P. vivax *in Culture.*

Here we are studying a parasite in which all stages from small rings up to full sporulation may be seen in the peripheral blood. Sporulation does not necessarily occur in the internal organs, and it is quite usual to find these forms in ordinary peripheral blood smears. When cultivating this parasite, therefore, it is important to draw the blood from the patient when the young forms predominate, so that it is then certain whether or not we are obtaining further development in the culture tubes. In the culture illustrated in Plate 10, figs. 21–30, we obtained the blood when young rings (fig. 21) predominated and no segmenting forms were found. After eight hours' incubation at a temperature of 39° C., a marked increase in size of the parasites was noted (figs. 22, 23, and 24), and pigment was now evident, being scattered throughout the protoplasm in fine granules. This arrangement of the pigment in *P. vivax* is in marked contrast to what occurs in *P. falciparum*, where the pigment always becomes arranged into a dense circular mass from the commencement. After 20–29 hours' incubation (figs. 25–30) sporulation was seen at different stages, the pigment being collected into a loose mass of granules in the centre of the parasite. Fig. 29

shows a parasite with 15 daughter-cells, and we have found on several occasions 16 spores, which we think is the usual maximum of daughter-cells found in *P. vivax*, although again we find all stages, from 2 spores up to 16, according to the stage of development at which we examine the cultures. We are quite certain that the spores are never so numerous as in the malignant tertian parasite. In these cultures clumping has not been found, and this explains why the parasites do not tend to be arrested in the internal organs during sporulation, although when full grown they are much larger than the malignant tertian parasite.

The absence of clumping in the case of the benign tertian parasite explains satisfactorily the absence of pernicious symptoms in this infection, and hence the absence of comatose malaria, and it also explains why all stages of this parasite are found in the peripheral blood, even up to sporulation.

The Morphology of the Benign Tertian Parasite in the Human Host.

This, again, is identical with that which we have just described under morphology in the culture tubes, figs. 21–30. The chief features in which this parasite differs from *P. falciparum* are the large size of the containing corpuscle, with the presence of Schüffner's dots, the straggling form of the medium-sized parasites, the scattered pigment in the presegmenting stages, and the smaller number of spores. These spores are larger than the spores of the malignant tertian parasite. The pigment in the sporulating forms is collected into a loose mass. With regard to the number of spores produced by this parasite we have found as many as 24, but the most usual number is 16–18. In culture the largest number we obtained on three occasions was 18. The following are the numbers of spores produced by *P. vivax* in the human host, according to several observers: Ross (1910) quotes the figures of Grassi and Feletti, 15–20 spores; Marchiafava, Bignami, and Mannaberg (1894), 16 spores; Golgi quotes 14–19 spores; Stephens and Christophers (1908), 15 or more spores; Deaderick (1910) gives 12–26 spores oftenest 16 spores.

In our opinion Deaderick's figures are most correct. All stages of this parasite are found in the peripheral blood, and the phenomenon of clumping has never been observed. Ross has taught recently that there are four splits with this parasite, that is 16 spores; three splits with the quartan parasites, that is 8 spores; and five splits, or 32 spores, with the malignant parasite.

Summary.

(1) The malignant tertian parasite has been successfully cultivated after the method of Bass and Johns on twelve occasions, and the benign tertian on three.

(2) It is unnecessary to remove the leucocytes from the blood before incubation. The optimum temperature would appear to be about 38° C., and the parasites may grow successfully at a temperature as low as 36° or 37° C.

(3) The time required for the full development of the parasite *in vitro* varies, but this variation is partly due to the age of the parasite at the time of incubation.

(4) The cultures of benign tertian differed from those of malignant tertian in that there was no tendency to clumping of the parasites in the former, either before or during sporulation.

(5) This difference appears to us to explain in a satisfactory manner why only young forms of malignant tertian are found in the peripheral blood, as the clumping tendency of the larger forms causes them to be arrested in the finer capillaries of the internal organs. It also explains the tendency to pernicious symptoms, such as coma, in malignant tertian malaria. All stages of the benign tertian parasite are found in the peripheral blood, and there are seldom pernicious symptoms, because there is no tendency to clumping.

(6) The malignant tertian parasite (*P. falciparum*) is capable of producing in maximum segmentation, 32 spores. On the other hand, benign tertian (*P. vivax*) produces, as a rule, during maximum segmentation, 16 spores; sometimes more may be produced, but the number is never 32.

(7) The pigment in *P. falciparum* collects into a definite, circular, and very compact mass early in the growth of the parasite. On the other hand, during the growth of *P. vivax* the pigment remains scattered in definite granules throughout the body of the parasite, till just before segmentation, when it collects into a loose mass of granules in the centre of the full-grown *Plasmodium*.

(8) The morphology of *P. falciparum* and *P. vivax* in the human host is identical with the morphology of these parasites as obtained in the culture tube.

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EXPLANATION OF PLATE 10.

All the figures in this plate represent the growth of the parasites in the culture tube. Magnification 1600 diameters.

Fig. 1 is a malignant tertian parasite at the time of inoculation of the culture tube. Corpuscle is shrunken; no pigment is seen, and no stippling of the corpuscle.

Fig. 2 represents 12 hours' growth at 36° C. No pigment is yet visible.

Fig. 3 represents 23 hours' growth at 41° C. Pigment is not yet evident, but the parasite has increased in size.

Figs. 4 and 5 represent 36 hours' growth; note the appearance of a compact mass of pigment. Fig. 5 is a double parasite.

Figs. 6 and 7 show commencing segmentation after 47 hours' incubation. Fig. 6 shows 5 daughter-cells, and fig. 7 20 spores.

Figs. 8, 9, and 10 show sporulating forms after 51 hours' incubation.

Fig. 11 shows complete sporulation (32 spores) after 52 hours' incubation. The containing corpuscle has burst and liberated the spores.

Fig. 12 was obtained after 56 hours' incubation.

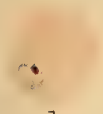
Fig. 13 represents a young merozoite of the second generation which has entered a new corpuscle, after 75 hours' incubation; note that there is no vacuole. The parasite is only 1·5 μ in its longest diameter.

Fig. 14 represents sporulation of the second generation after 3 days' incubation.

Fig. 15 shows a young merozoite of the third generation after 4½ days' incubation.

Fig. 16 represents sporulation in the third generation after 6 days' incubation; only 8 spores have formed.

Fig. 17 is a young merozoite of the fourth generation after 7 days' incubation.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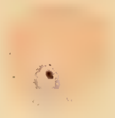
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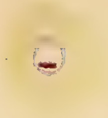
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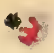
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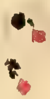
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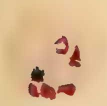
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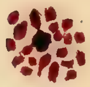
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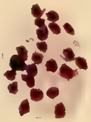
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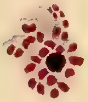
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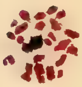
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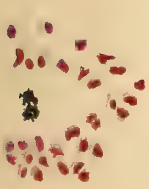
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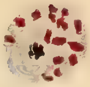
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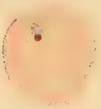
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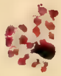
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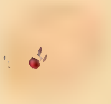
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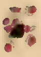
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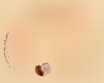
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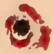
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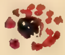
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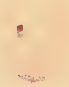
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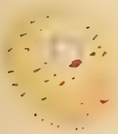
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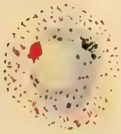
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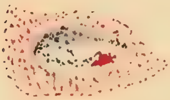
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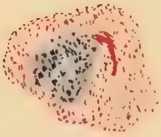
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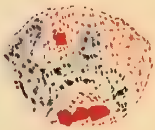
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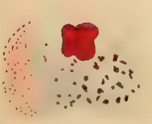
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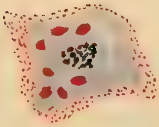
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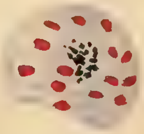
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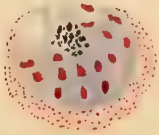
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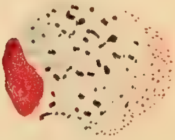
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J. G. Thomson & D. Thomson, del.

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Fig. 18. Eight days' incubation, showing commencing segmentation in the fourth generation.

Fig. 19. Nine days' incubation, showing sporulation in the fourth generation.

Fig. 20 shows a young merozoite of the fifth generation after 10 days' incubation.

All of the above figures were obtained from one culture tube, without the removal of leucocytes and without the addition of fresh serum or corpuscles.

Fig. 21 shows a young benign tertian parasite at the time of inoculation of the culture tube.

Figs. 22, 23, and 24 represent 8 hours' growth in culture tube at 39° C.

Figs. 25–30 show parasites obtained from the culture tube after 20–29 hours' incubation.

Fig. 30 is a female gamete (undivided chromatin and scattered pigment) found in the culture tube; similar gametes were found in the blood at the time of inoculation of the cultures, so it is probable that this gamete was one of these, and that it had not developed in the culture.

Note the large size of the corpuscles in the benign tertian as compared with those in the malignant tertian, also the Schüffner's dots and the scattered pigment. The spores also are larger and fewer in number in the case of the benign tertian parasites.

CROONIAN LECTURE: *The Origin of Mammals.*

By Dr. ROBERT BROOM.

(Lecture delivered June 5, 1913.)

(Abstract.)

An endeavour is made to trace the evolution of mammals from Cotylosaurian ancestors through the carnivorous Therapsida. In Upper Carboniferous times the line probably passed through some primitive generalised Pelycosaurs; in Lower Permian through primitive, probably Therocephalian, Therapsids. In Middle and Upper Permian the line passed through the Gorgonopsia. In Triassic times the mammalian ancestors were small generalised Cynodonts. In Lower Jurassic the mammals are so Cynodont-like, and the Cynodonts so mammal-like, that in no single case are we absolutely certain which is which.

In the Therocephalia, the Gorgonopsia, and the Cynodontia, the skull is very mammal-like. The zygomatic arch is, as in mammals, formed by the jugal and the squamosal. The teeth are divided into incisors, canines and molars. In the later Gorgonopsians there is an imperfect secondary palate: in Cynodonts a complete secondary palate as in mammals. In Permian Therapsids there is a single occipital condyle; in the Triassic Cynodonts

there may be a single condyle slightly divided or two exoccipital condyles. There is, on passing from earlier to later types, a steady increase in the size of the dentary and decrease in the size of the other elements of the jaw. The quadrate also becomes much reduced in the higher types. In Gorgonopsians and probably all earlier types the arch of the atlas is a pair of bones; in Cynodonts, as in mammals, there is a single arch.

It is argued that the small Gorgonopsians fed almost exclusively on the comparatively slow-moving, small, herbivorous Anomodonts. In the Trias the small Anomodonts became very rare, and the carnivorous Therapsids had to feed on other small forms, apparently the more active lizard-like Cotylosaurs, such as *Procolophon.* The change of habit resulted in the Cynodontia.

In Upper Triassic times the larger Cynodonts preyed upon the large Anomodont, *Kannemeyeria*, and carried on their existence so long as these Anomodonts survived, but died out with them about the end of the Trias or in Rhætic times. The small Cynodonts, having neither small Anomodonts nor small Cotylosaurs to feed on, were forced to hunt the very active long-limbed Thecodonts. The greatly increased activity brought about that series of changes which formed the mammals—the flexible skin with hair, the four-chambered heart and warm blood, the loose jaw with teeth for mastication, an increased development of tactile sensation and a great increase of cerebrum. Not improbably the attacks of the newly-evolved Cynodont or mammalian type brought about a corresponding evolution in the Pseudosuchian Thecodonts which ultimately resulted in the formation of Dinosaurs and Birds.

A Case of Abnormal Trichromatic Colour Vision due to a Shift in the Spectrum of the Green-Sensation Curve.

By Sir W. de W. Abney, K.C.B., F.R.S., and W. Watson, D.Sc., F.R.S.

(Received June 13,—Read June 26, 1913.)

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The Trypanosomes causing Dourine (Mal de Coït *or* Beschälseuche).

By B. Blacklock, M.D., and Warrington Yorke, M.D.

(Communicated by Sir Ronald Ross, K.C.B., F.R.S. Received May 19, 1913.)

(From the Runcorn Research Laboratory, Liverpool School of Tropical Medicine.)

[Plate 11.]

About the middle of the year 1910, whilst examining our laboratory strain of *Trypanosoma equiperdum*, short parasites, certain of which exhibited a posterior displacement of the nucleus, were observed. This strain had been preserved at the Runcorn Laboratory by passage through experimental animals since 1907, when it was obtained from the Kaiserliches Gesundheitsamt, Berlin. As these posterior nuclear forms had not been described in *T. equiperdum* we asked Prof. Schilling for further animals infected with this strain which had been preserved by him. Examination of the parasites in these animals likewise revealed the presence of the posterior nuclear forms.

Having satisfied ourselves that these forms are a constant feature of the strain, we published a short preliminary note* drawing attention to this fact.

As previously *T. equiperdum* had always been described as a monomorphic parasite invariably furnished with a free flagellum, we decided to examine for ourselves various strains of this parasite preserved at the present time in the European laboratories. We were enabled to collect the following three strains of the trypanosome.

Strain A.—Berlin strain. This is our old laboratory strain. Prof. Schuberg informs us that this was the trypanosome used by Uhlenhuth, Hübener, and Woithe† in their experimental work on Dourine in 1908. These authors state that the virus was obtained from a horse suffering from Dourine brought over to Germany from Algiers for experimental purposes by the firm of Hagenbeck. The work of Salvin Moore and Breinl,‡ and others, on *T. equiperdum*, was done with this strain.

Strain B.—Frankfurt strain. This strain was obtained in 1906 from Prof. Ehrlich. Unfortunately, no record of its origin is available, but

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* Yorke, W., and Blacklock, B., "A Note on the Morphology of a Strain of *T. equiperdum*," 'Brit. Med. Journ.,' August 31, 1912.

† "Experimentelle Untersuchungen über Dourine," 'Arb. a. d. Kaiserl. Gesund.,' 1907, vol. 27, p. 256.

‡ "Life History of *T. equiperdum*," 'Roy. Soc. Proc.,' 1908, B, vol. 80, p. 288.

Prof. Ehrlich informs us that there is no doubt that he obtained it from a trustworthy source.

Strain C.—East Prussian strain. This strain was supplied to us by the veterinary department of the Kaiserliches Gesundheitsamt. It is the virus responsible for the East Prussian outbreak of Beschälseuche in horses in 1905.

We have made a careful study of the morphology of each of these three strains. For the sake of comparison the infection in rats and guinea-pigs has been observed in each case. On examining the blood of infected guinea-pigs we were at once impressed by the large number of short, aflagellar forms occurring in Strain A as compared with B and C.

To give mathematical expression to this statement 20,000 individuals of each strain were examined regarding the presence or absence of a free flagellum. The examinations were conducted by observing from 200 to 1000 individuals on different days of the infection in both rats and guinea-pigs.

A glance at Table I will show that the impression—that Strain A is different from Strains B and C—conveyed by casual examination of blood films of guinea-pigs infected with each of these strains is correct, for whereas of the 20,000 individuals observed in each strain, 1321 aflagellar and spicule* forms were encountered in Strain A, only 18 and 86 similar forms were seen in

Table I.—Showing the Number of Parasites of Various Types encountered in 20,000 of each of the Three Strains.

Strain.	Animal.	Number observed.	Forms with free flagellum.	Aflagellar forms.	Spicule forms or forms with very short flagellum.	Posterior nuclear forms.
A	Guinea-pig ...	10,000	8,814	378	808	332
	Rat	10,000	9,865	30	105	128
	Total ...	20,000	18,679	408	913	460
B	Guinea-pig ...	10,000	9,986	0	14	0
	Rat	10,000	9,996	1	3	0
	Total ...	20,000	19,982	1	17	0
C	Guinea-pig ...	10,000	9,918	22	60	0
	Rat	10,000	9,996	0	4	0
	Total ...	20,000	19,914	22	64	0

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* By these forms we mean parasites in which the flagellum is free to the extent of about 1 μ or less.

Strains B and C respectively. It is to be observed that this distinction between the strains is much more clearly brought out by an examination of guinea-pigs than of rats.

Applying the present method of classification, this morphological distinction is at once sufficient to differentiate Strain A from the others. If we adopt Laveran's recent scheme for the classification of pathogenic trypanosomes, Strain A belongs to Group 3 (in which certain individuals have a free flagellum, whilst others have not), whereas Strains B and C must be classed amongst the trypanosomes forming Group 1 (those in which all the individuals have a free flagellum).

Moreover, there is a further morphological peculiarity by which Strain A is differentiated from Strains B and C. As is described in our previous paper (*loc. cit.*), certain of the short forms of the former strain exhibit a posterior nucleus. The phenomenon, so far as we have been able to ascertain, is limited to this strain and does not occur in either of the other two.

Having satisfied ourselves that Strain A was morphologically distinct from Strains B and C, we examined more minutely the morphology of the two latter strains with a view to ascertaining whether these are different one from the other or whether they are identical.

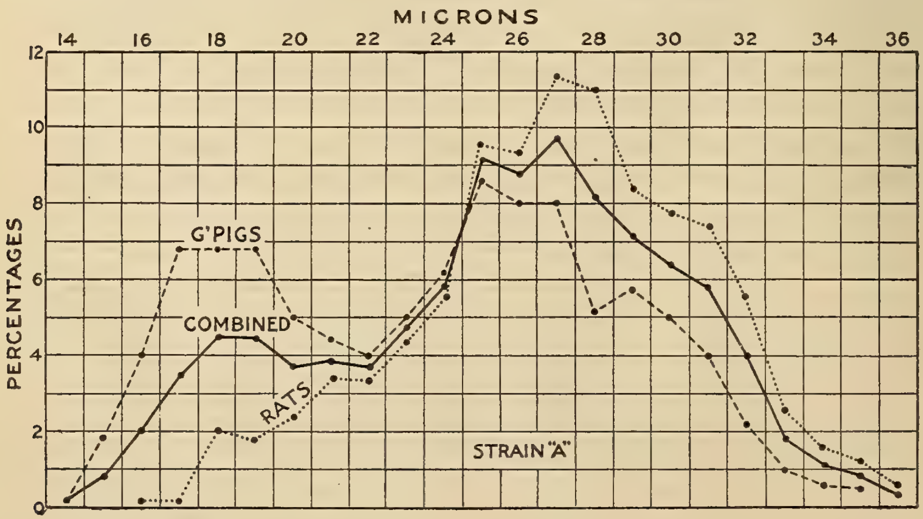
Attention has already been called to the fact that in both the strains long free flagellated forms constitute the vast majority of the parasites encountered. There is, however, a point to which we must refer. In the East Prussian strain the cytoplasm is continued in many individuals a considerable distance along the flagellum, so that even the longest forms encountered have frequently a comparatively short portion of flagellum free. Most of the aflagellar or spicule forms in this strain are not "short stumpy" forms properly speaking, but medium or even long forms in which the cytoplasm is carried along the flagellum. A few short forms, concerning certain of which some doubt existed as to whether or not there was a free flagellum, spicule forms, were met with in both strains B and C. These, however, may be neglected, as at least an equal number of short forms with a questionable free flagellum is seen in *T. evansi*, which is recognised as the type of the trypanosomes constituting Group 1 in Laveran's classification.

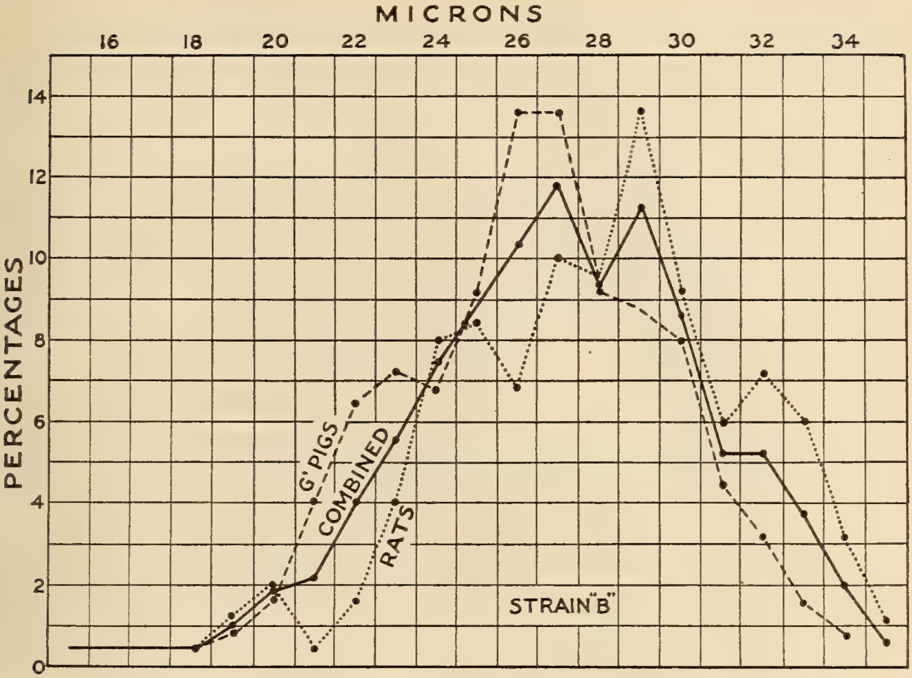
In Table II are given the dimensions of the trypanosomes of each of the three strains in rats and guinea-pigs. But little assistance in the differentiation of the strains is to be obtained from these figures. It is to be noted that in each case the average dimensions of the parasites in the rat are greater than those of the same strain in guinea-pigs. This difference, which is but slight in Strains B and C, is more marked in the case of Strain A.

Table II.—Giving the Maximum, Minimum, and Average Lengths of each of the Three Strains.

Strain.	Animal.	Number of individuals measured.	Maximum length.	Minimum length.	Average length.
A	Rat	500	36·0	16·0	26·9
	Guinea-pig	500	35·5	14·5	23·7
	Total	1000	36·0	14·5	25·3
B	Rat	250	35·0	15·5	27·7
	Guinea-pig	250	34·0	15·5	26·3
	Total	500	35·0	15·5	27·0
C	Rat	250	33·0	17·5	25·6
	Guinea-pig	250	32·0	18·0	24·8
	Total	500	33·0	17·5	25·2

A survey of the curves obtained by plotting out in percentages the various lengths of trypanosomes encountered in each of the three strains is of interest. It will be observed that in the case of rats the curves of each of the strains correspond fairly closely. They are those of monomorphic trypanosomes, the main peak in Strains A and C being at 27, whilst in Strain B it is found at 29μ. A striking difference, however, is to be observed in the graphs compiled from the measurement of the parasites in guinea-pigs, for whereas in




MICRONS
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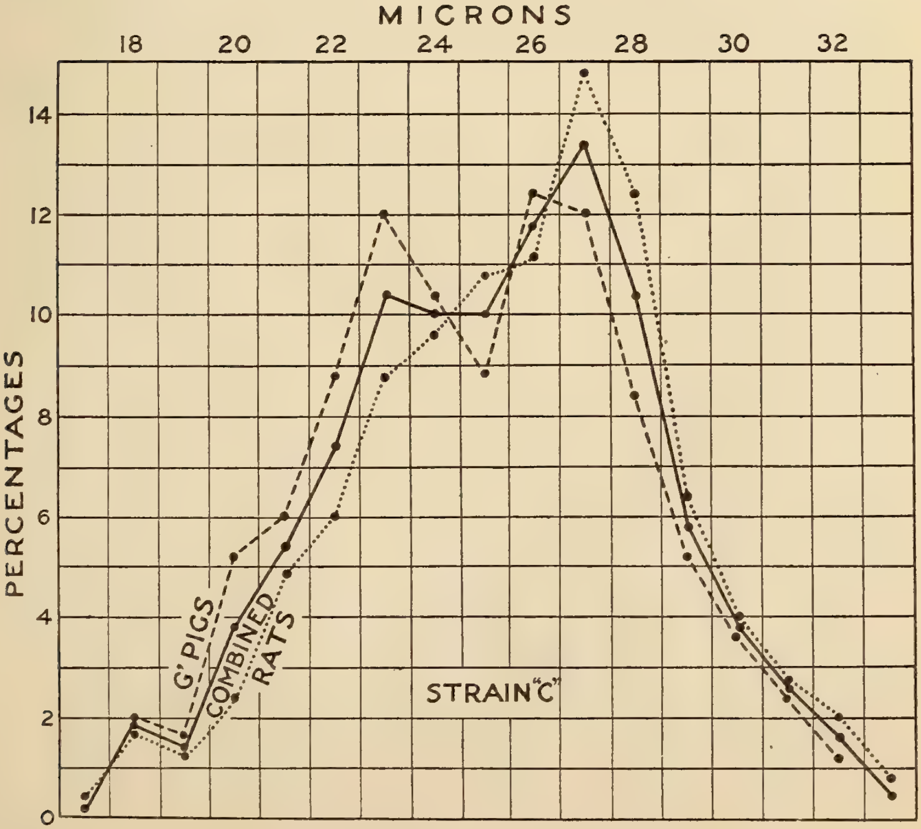
RATS
STRAIN "B"


MICRONS
18
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32
PERCENTAGES
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4
6
8
10
12
14
G' PIGS
COMBINED
RATS
STRAIN "C"

Strains B and C the curves are again those of monomorphic trypanosomes and correspond closely to those obtained from the rats, in Strain A the curve has two widely separated main peaks, one occurring at 17–19μ and the other at 25–27μ, and is typically that of a dimorphic parasite. Apart then from the slightly greater average length of Strain B and the prolongation of the cytoplasm along the flagellum in Strain C noted above, we have been unable to discover any character which enables us to distinguish these two strains. Both, however, are differentiated from Strain A by the presence in the latter of posterior nuclear forms and by the occurrence of typical "short stumpy" forms. These distinctive points are more readily brought out by a study of the infections in guinea-pigs than in rats.

Table III.—Percentage of Short, Intermediate, and Long Forms in each of the Three Strains.

Strain.	Animal.	Short and stumpy, 14–21 μ.	Intermediate, 22–24 μ.	Long, 25–36 μ.
A	Rat	10·0	13·4	76·6
	Guinea-pig	35·8	15·2	49·0
	Total	22·9	14·3	62·8
B	Rat	5·2	13·6	81·2
	Guinea-pig	7·2	20·4	72·4
	Total	6·2	17·0	76·8
C	Rat	10·4	24·4	65·2
	Guinea-pig	14·8	31·2	54·0
	Total	12·6	27·8	59·6

In view of these facts we must reconsider the nomenclature of the parasites and decide which of them corresponds with the type to which the name *T. equiperdum* was given. In order to determine this point it is necessary to refer to the literature of the subject.

In 1896, Rouget* described a trypanosome in the blood of an Algerian horse which was suffering from Dourine. Rouget succeeded in infecting a number of laboratory animals with the parasite, but, unfortunately, the strain was lost shortly afterwards. Three years later (1899) Schneider and

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* "Contribution à l'Étude du Trypanosome des Mammifères," 'Annales de l'Institut Pasteur,' 1896, vol. 10, p. 716.

Buffard[*] found a trypanosome in two Algerian horses and an ass which had Dourine. They succeeded in reproducing Dourine in a horse experimentally after passage of the virus through a dog. This work was subsequently confirmed by Nocard.[†]

Doflein,[‡] in his book on the parasitic protozoa, 1901, refers briefly to Rouget's description of the disease and to the morphology and pathogenicity of the parasite. The photographs illustrating Doflein's article are apparently reproductions of Rouget's original plates. Reference is made to Nocard's work on the transmission of the disease by coitus, and also to the fact that he believed the parasite to be identical with those causing Nagana and Surra. Since, however, the disease was spread by coitus and the trypanosome failed to infect ruminants, Doflein considered that the parasite described by Rouget was distinct from those causing Nagana and Surra and gave to it the name *T. equiperdum.* It appears, therefore, that five years after the strain was lost Doflein named the parasite purely on the description of the trypanosome given by Rouget. Rouget's account of the morphology of the parasite must then be regarded as the authentic description of *T. equiperdum.* Unfortunately, in Rouget's original paper (*loc. cit.*) the account of the morphology of the trypanosome is rather vague. Its length is given as 18–26μ and its breadth as 2–2·5μ. The parasite is described as terminating in a free flagellum which forms about a fourth of the total length of the creature.

All subsequent authors agree that *T. equiperdum* is a monomorphic parasite in which all forms are furnished with a free flagellum. The Frankfurt and the East Prussian strains conform to this description. The strain brought from Algiers by Hagenbeck and maintained at Berlin and here, under the name *T. equiperdum,* differs in important particulars from the classical description of *T. equiperdum,* and from the other strains we have examined. We must conclude, therefore, that the symptom-complex of the disease clinically known as Dourine can be produced by more than one species of trypanosome.

It is important to record that we are unable to distinguish morphologically the parasite of Hagenbeck's Dourine horse from *T. rhodesiense, T. pecaudi,* or *T. ugandæ* (*T. brucei* of Uganda). Nevertheless, we hesitate to suggest that it is identical with any, or all, of these, in view of the fact that it produced in a horse symptoms clinically known as Dourine. That a trypanosome indistinguishable morphologically from *T. rhodesiense* should be disseminated amongst horses by coitus is of considerable interest. We are at present conducting experiments with a view to ascertaining whether these three strains, all of them obtained from horses suffering from "mal de coït," are still capable, after numerous passages through laboratory animals extending over many years, of being transmitted in equines by coitus. For the present, we propose for this *rhodesiense*-like trypanosome the name *T. equi.*

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[*] "Le Trypanosome de la Dourine," 'Archives de Parasitologie,' 1900, vol. 3, p. 124.

[†] "Sur les Rapports qui existent entre la Dourine et le Surra ou le Nagana," 'Compt. Rend. Soc. Biol.,' 1901, vol. 53, p. 464.

[‡] 'Die Protozoen als Parasiten und Krankheitserreger,' Aufl. I, p. 66, Jena.

DESCRIPTION OF PLATE.

Drawn with Abbé camera lucida, using 2 mm. apochromatic objective and No. 12 compensating ocular (Zeiss). Magnification 2000 diameters.

Figs. 1- 6.—Strain A (Berlin Strain).
Figs. 7-10.—Strain B (Frankfurt Strain).
Figs. 11-14.—Strain C (East Prussian Strain).

Studies in the Heat-production Associated with Muscular Work. (Preliminary Communication: Section A.—Methods; Section B.—Results.)

By J. S. Macdonald, University of Sheffield.

(Communicated by Prof. C. S. Sherrington, F.R.S. Received June 14,—Read June 26, 1913.)

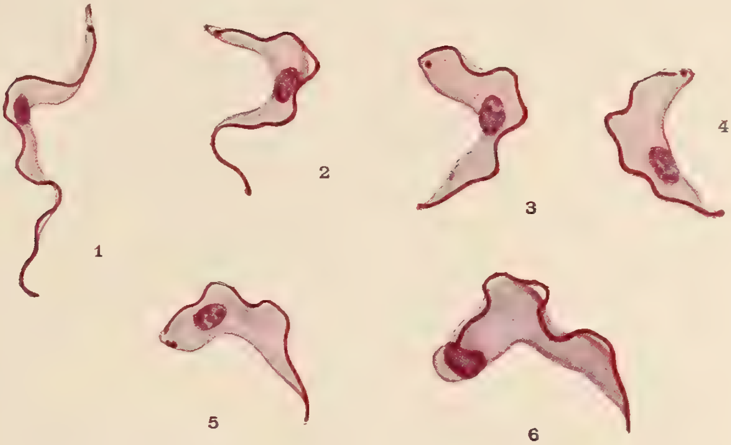
Section A.—Method.

The calorimeter with which the included data have been obtained was built upon the plan described by Benedict,[*] omitting, however, such parts as were essential rather to a study of the respiratory gases than to measurements of heat-production. The general principles of its construction are well known, exceedingly ingenious, were developed by Atwater and Benedict, and are briefly as follows: The body of the calorimeter is of sheet copper built upon an external wooden framework, on which again is built externally an outer zinc box enclosing, but nowhere in contact with, the calorimeter box proper. Between the two metal boxes, sets of thermocouples arranged in groups are utilised to discover any differences of temperature likely to lead to a radiation of heat from one box to the other across the intervening air space partially occupied by the wooden framework. In the walls of a still

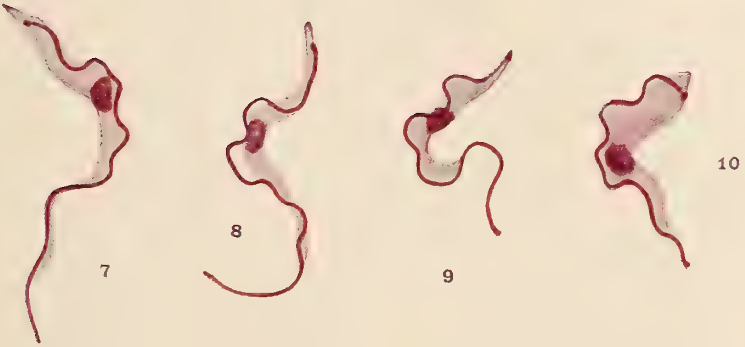
[*] 'A Respiration Calorimeter, etc.,' published by the Carnegie Institution of Washington, 1905.

Blacklock & Yorke.

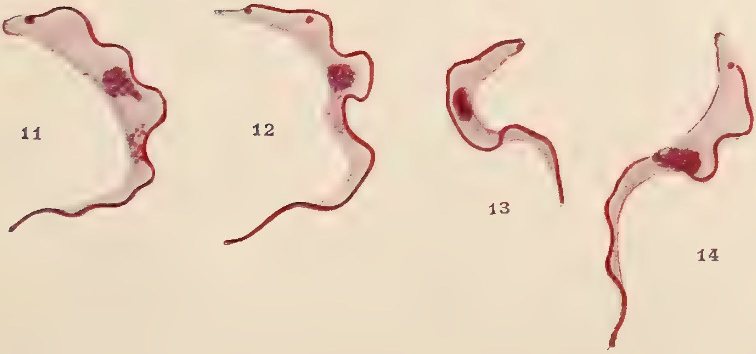
Roy. Soc. Proc. B Vol. 87. Pl. 11



Strain A



Strain B



Strain C

A. M. Brookfield del.

Crout sc. & imp.

more external wooden shell are placed means by which heat may be added to or subtracted from the zinc box in a graduated fashion so as to annul any such observed differences in temperature. Thus the zinc box is kept in each of its several zones, each zone corresponding to a group of thermocouples, at the same temperature as the copper box, and the thermal insulation of the calorimeter is thus insured.

The subject of the experiment enters the calorimeter by a window space left in the walls of this nest of boxes, and is then sealed in by glass and wax. The heat produced in his body, as well as the heat into which all his mechanical work is finally converted, raises the temperature (1) of an insulated radiator system through which a steady stream of water is maintained; (2) of the calorimeter box; (3) causes some evaporation of water from his respiratory passages and skin, and (4) tends to raise his own temperature. Each one of these four stores of heat is observed in suitable ways, and the summed account of their alterations provides a measure of the heat-production of, or total transformation of energy in, the subject.

Now, although the main principles of construction are the same as those of the original calorimeter in the Middletown University, I have altered many small details, in part for the sake of economy, in part for convenience, and in some small degree with a view to improvement. Most of these I hope to describe briefly in a more extended communication; to one alone, as somewhat modifying the usage of the instrument, I refer at present. This modification consists in the introduction of a source of heat other than the man, in some cases adding substantially to the total heat-production. The main reason for this change in procedure was a desire to follow the progress of changes in the heat-production of the subject more closely than was possible with the original arrangement.

Whilst endeavouring to follow the events of shorter periods of time, *e.g.*, complete observations every 5 minutes, it was soon found that of the four stores into which the heat produced by the subject was delivered, one alone, the temperature of the calorimeter, had a sufficiently elastic capacity to follow abrupt changes. Associated, however, with the possession of this advantage was the failing that, thus abruptly changed, it tended to form a similarly abruptly changing site of heat leakage, unless rapidly checked by adequate and almost simultaneous adjustments in the temperature of its environment, that is of the enclosing zinc box. On this account it is necessary that modifications in heat-production must be kept well within the limits of adjustment of this process, which by the way may conveniently be termed the balancing of the calorimeter. It is only when such limits are not transgressed that any reliance can be placed upon the value of such corrections for changes in the temperature of the calorimeter as may be ascertained by appropriate calibration experiments. I have therefore placed five incandescent lamps within the calorimeter, reading the power absorbed by them by means of a wattmeter, and changing this value by means of suitable resistances. In such a way it is possible to compensate alterations in the heat-production of the subject, and to dispense with the necessity for any but the smallest adjustments in the balance of the calorimeter. It is clear that such a process of compensation may be managed with greater abruptness and precision than complicated modifications in the balance, using the constantly observed temperature of the calorimeter as an index of success or failure.

In addition to the subject and these lamps two other sources of heat have been present within the calorimeter during these experiments. Thus, in the first place, there is the power-absorption of electromagnets forming part of an electrical brake applied to the cycle-ergometer upon which the subject performs definite amounts of mechanical work, and this is followed by a voltmeter and ammeter arranged in this circuit. Two such electromagnets placed in separate positions each environ a part of the path traversed by the periphery of a copper disc by which the hind wheel of the cycle has been replaced. Benedict has recently described the calibration of such an instrument provided with a single electromagnet and with a thicker copper disc. He is fortunate in possessing in this machine an instrument in which, at a certain useful revolution rate, small changes of speed occasion directly proportionate variations in the amount of mechanical work performed. This useful property has been found by Benedict and Cady to be attributable to the reaction of the eddy-currents in the copper disc upon the value of the electromagnet exciting their presence, and is dependent upon the dimensions of the poles of the magnet and upon the thickness of the copper disc and its resistance. I am not so fortunate, and the mechanical work performed on my cycle varies more rapidly, rising with the square of the revolution rate. In this case it should be noted that the maintenance of a uniform speed is of greater importance.

To return to the sources of heat within the calorimeter, I have in the second place to mention the power-absorption due to a fairly powerful fan placed within the calorimeter and maintaining there the conditions of a moderate breeze. Here again a voltmeter and ammeter are used to assess the heat-production due to this source. This value added to that due to the brake and to that due to the incandescent lamps provide a "subtraction" which has to be taken from the total heat measured at any time before the heat-production of the subject is known.

Now I am aware of the fact that each of these additional sources of heat is also an additional source of error, subtracting to some degree from the theoretical precision of the instrument and diminishing its value as gauged by the measurement of any standard source of heat. In the practical usage of the instrument as applied to varying sources of heat they have a great value, which, in my opinion, fully justifies their insertion. Of the value of the lamps I have already spoken. The fan is used to keep the air inside the calorimeter in a state of satisfactory admixture, so that samples withdrawn from it in the general air-current are given a value in reference to the general air within the calorimeter of a simple and easily determined kind, and may thus be used to estimate changes in the value of this air. In these particular experiments this point is only of importance in dealing with the storage of aqueous vapour within the calorimeter.

Air is made to enter the calorimeter through a tube guarded by thermocouples, a connected set being placed in the exit tube. Thus any difference between the temperature of the entering and leaving air is known. Means for warming the entering air are provided, but I have not so far made any arrangements for cooling it when necessary. The adjustment of these temperatures has not then always been as good as might be, but they are also observed by means of mercurial thermometers and any observed difference allowed for.

Air is sucked out of the calorimeter by a rotary pump at a measured rate, varying in these experiments from 300 to 450 cubic feet per hour. In the tube forming the path of the leaving air, and in the similar tube for the entering air, a dilatation is provided in the form of an interpolated glass box. In these glass boxes "wet and dry bulb" thermometers are placed, the stems outside for observation, so that an estimate may be formed of the excess aqueous vapour derived from the calorimeter. In my earlier experiments the water-vapour was weighed after absorption by sulphuric acid, or rather a fraction obtained from a section of the air-path was thus treated, but this plan was abandoned after comparative trial, mainly as not lending itself so well to the observation of five-minute periods of heat-production. Here perhaps the interests of absolute precision have apparently suffered from the desire to study the events of shorter periods of time.

Much more important than this path of heat escape with the aqueous vapour of the air current is the main path which issues along the stream of water from the internal radiator system. This radiator system, originally distributed along the line of junction of the walls and roof of the calorimeter, I have extended so as to be co-extensive with the roof of the calorimeter, beneath which it is suspended in an insulated fashion. The entering water is driven to it from a constant-pressure supply through coils of tubing situated in ice-filled tanks, and arrives at a temperature sometimes less, sometimes greater, than 5° C. It passes out into a balance, such as that described by Benedict. This balance is duplicate, and automatic arrangements provide that the filling of the collecting pan suspended from one beam shall immediately be followed by admission of the stream of water into the similar collecting pan suspended from the second beam. The change over is made known by the ringing of an electric bell, and the weight then ascertained by the observer thus warned. Thus, this is the only set of readings which is not arranged in five-minute periods, and average rates of flow have to be accepted for each of the included five-minute periods. The intervals of such readings have varied from 12 to 20 minutes.

The remaining readings are taken by the observer every time warning is given by a bell attached to a five-minutes clock, in order somewhat as follows:—

(*a*) The wet and dry bulbs in the entering and leaving air.

(*b*) The thermometers in the entering and leaving water.

(*c*) The resistance of some 570 ohms of iron wire arranged on a series of coils within the calorimeter, by means of which its temperature is assessed.

(*d*) The number of revolutions of the cycle as read upon a cyclometer driven by an electromagnet from contacts on the cycle.

(*e*) The watts due to the lamps (wattmeter).

(*f*) Voltmeters and ammeters connected with the circuits of the fan and of the cycle-brake.

(*g*) The surface temperature; and

(*h*) the rectal temperature of the subject, as evidenced by galvanometric deflections due to suitable thermocouples.

(*i*) The temperature of an incubator in which are placed the "constant temperature junctions" of these thermocouples.

The data collected in this way have been dealt with on a uniform plan throughout the whole series of experiments here included. The only details of this plan which perhaps should be dealt with briefly here are (1) the means of applying the correction for the temperature of the calorimeter, and (2) for estimating the storage value of the air space in the calorimeter.

By suitable calibration experiments it has been found that an alteration of 0·01 ohm in the resistance of the calorimeter "thermometer" is equivalent to an addition, or subtraction, of 1/6 kilo-calorie (or kalorie, as written elsewhere in this paper). Now, in making up my accounts, I have expressed all the results in rates of change per hour, so that a change of this value observed in a five-minutes period (1/6 kalorie gained or lost in five minutes) is expressed as a change of 2 kalories per hour in the rate of heat-production. Measurements of the resistance of a sample of the iron wire used in the construction of the calorimeter "thermometer" were carefully undertaken by Dr. Chapman, to whom, in the earlier stages of the construction and calibration of the calorimeter, I am indebted for a considerable amount of help. It was then found that a change of 1° C. in the temperature of this wire was accompanied by a change in the resistance such as, applied to the amount of wire in the calorimeter, would correspond with a change of 2·54 ohms in resistance; and this, seeing that 0·01 ohm involves a change of 1/6 kalorie, would involve a change of 42·3 kalories. Thus the thermal "water equivalent" of the calorimeter would seem to have the high value of 42·3 litres.

As to the storage space within the calorimeter, that is a figure which, for the present, is of much less importance, since the data given are taken from a time of work-performance when such storage is practically at an end. The storage space is, as we have found, less than the total internal air space. This fact has been ascertained by my assistant, Dr. Duffield, in calibration experiments, in which carbonic acid was delivered into the calorimeter at a definite rate, and comparison was made between this rate of entrance and its observed rate of departure in the air current. These experiments are in progress and will be described later by Dr. Duffield. In the meantime, for the purposes of this communication, I have taken the storage space as less than the total space by a quantity equivalent to that of the air entering over a single five-minute period. It is probable that later I may have to increase this subtraction, but the relative unimportance of the point may be judged from the values given below. These are the average figures provided by the set of experiments described as "Group D" in the next section, and from them it is possible to see how small the figures for the storage of aqueous vapour are, and how little their modification will mean when the "storage space" is better known.

Data from Group D (see p. 109).	Kals.
(I) Heat carried away by the water-stream	303
(II) Heat carried away with the aqueous vapour of the air-stream	28
(III) Heat stored in the water-vapour of the calorimeter	2
(IV) Heat stored in the walls of the calorimeter (calorimeter temperature)	10
(V) Allowed for difference between the temperatures of the entering and leaving air	1
(VI) Allowed for change in the rectal temperature of the subject	2
Heat production	346

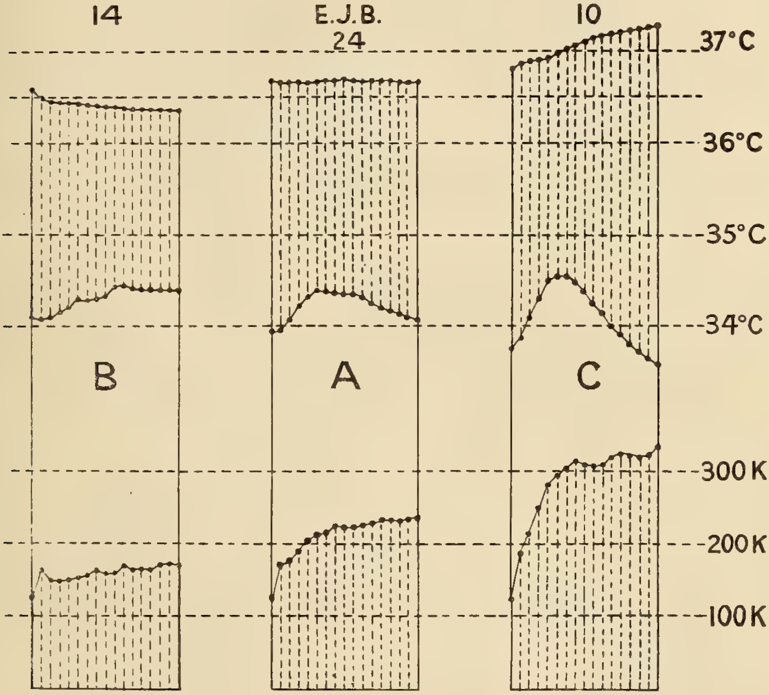
The staff at work upon the calorimeter in most of my earlier experiments consisted of two men; later, and in the whole of the experiments to which reference is made here, of three men. I must mention in the first place my laboratory assistant, Mr. A. Wallis, who has been a most valuable aid in the construction of the calorimeter and in the design and construction of accessory apparatus. In these experiments he has been responsible throughout for the balance of the calorimeter surface, and has kept records of the currents due to the various sets of thermocouples of very considerable importance to a discussion of the data. In the second place the observer, assisted by a note-taker, who recorded the observations as they were made and immediately dictated. At the present time this staff is increased by Dr. Duffield, who is now responsible for the gas-analysis side of the investigation. If I may forestall one of his measurements here, it is to record the fact that the air space of the calorimeter is 176 cubic feet. To obtain this measurement carbonic acid gas was delivered into the calorimeter, the enclosed atmosphere then thoroughly mixed by the fan, and its percentage content of CO_2 obtained from a sample. The rotary pump was then started, and the total amount of the gas ascertained as it was gradually withdrawn with the air current. The figure agrees very closely with that obtained by measurements of the average length, width, and depth of the calorimeter, but is of value because of uncertainty due to irregularities in the walls and because of a subtraction made necessary by the presence of the radiator system, etc.

A most important part of the method has been the securing of financial assistance, and in this connection I owe much to the British Association.

Section B.—Results.

The accompanying figure may serve to illustrate my need for considering the results of these experiments under two headings: (1) the events of the first hour of cycling; (2) the events of the second hour. Fig. A represents an average of data obtained in 24 separate experiments upon E. J. Briscoe at various known revolution rates of the cycle, and with various known values of the electrical brake, covering the whole range of work-performance upon the cycle which he could maintain uniformly for the required length of time. The lowermost curve in the figure is that of the heat-production; the middle curve gives his surface temperature; the uppermost is the rectal temperature. Fig. B is the average curve of such of these experiments—14 in number—as are below the general average of the full series. Fig. C is the average curve of the 10 experiments which lie above the general average of heat-production.

In each figure the different curves of each set (A, B, C) have the same meaning. Thus the lowermost always represents the heat-production, etc. Their examination will show that the experiments have been balanced so as to yield a general average such that in it the rectal temperature is practically uniform throughout, and is represented by what is almost a straight line (fig. A). It follows, therefore, that the underlying curve of heat-production is practically devoid of any corrections due to alterations in the temperature of the subject. Nor will any modification in my system for dealing with such corrections, nor any new and quite different system, affect this fact, so long as such systems are based upon observations of the rectal temperature. Now the importance of this point is this, that the curve of heat-production is seen gradually rising towards a maximum. Thus although mechanical work was being performed at a uniform rate throughout each experiment, and, therefore, at an average uniform rate throughout the whole series of experiments, it would appear that the "heat-production" or total amount of energy transformed which was associated with its performance was not uniform, but that it increased during the progress of work performance. There is, however, no confirmative evidence as to any state of affairs like this obtainable from investigations of the primary



chemical changes underlying this transformation of energy. This is true of such experiments in general, and in this particular case has been found to be true by Dr. Duffield, investigating the carbonic output in similar experiments in the calorimeter. There is little doubt that the curve of transformation of chemical energy has a much greater parallelism to that of work performance, and would, therefore, in this case be represented by a straight line. It follows then that my experimental curve of "heat-production" has only a secondary relation to the real curve of transformation of energy, and that I am losing sight of some part of the energy transformed, large at first, but subsequently diminishing. I find myself therefore obliged in dealing with the events of the first hour to consider the following possibilities:—

A. That a storage of heat in the disc of the cycle, representing some large fraction of the mechanical work done, is of great importance, and has been neglected.

B. That there is a storage of heat within the subject, as, for instance, within his musculature, to which the rectal temperature is no adequate index.

C. That some of the energy transformed in the oxidation processes accompanying the performance of work is stored in the body in some form other than heat.

Now, the first of these possibilities, dealing with the rising temperature of the copper disc of the cycle, is certainly a source of some error. It cannot, however, be considered as a source of errors of the magnitude present in this case, since at the most it could hardly account for more heat than is due to the conversion of the mechanical work performed, and this value is no more than a small fraction of the missing quantity. Obviously again, the third, and extremely interesting, possibility is best left on one side until the second one is adequately considered, namely, that under working conditions the rectal temperature fails to represent the real average temperature of the body. It has been noted that, during conditions of rest, the rectal temperature may be taken as an index to variations in the real average temperature, even if it is not on absolutely the same level as that average temperature, since the variations in the two are probably similar. Thus, it has been observed that temperature observations taken under such conditions at several different sites (*e.g.* skin and rectum) give parallel curves. In this case there is no such parallelism, as may be seen from the curves of rectal (uppermost) and surface (middle) given in the figures.

It is, indeed, probably the case that the rectal temperature represents no more than the mean temperature of the mixed blood sent from the heart to one part of the general circulatory system, a particular part in which, owing to an absence of much local performance of work, this mean temperature is only slightly changed, so that the mean temperature of the gut is much the same as that of the arterial blood entering it or of the venous blood leaving it. The mixed blood that is sent from the heart, however, may simply, and for the moment, be thought of as blood from the cooling district of the skin plus blood from the warm district of the musculature, and, whereas the blood from the skin may fairly represent the temperature of the mass that it traverses there, it is not at once obvious that the blood returning from the great bulk of the skeletal musculature, although of a quantity sufficient to cope with the demands of a greatly increased oxygen-transport, should at the same time be so increased as to keep the musculature at a temperature only slightly greater than its own. The rectal temperature may conceivably fail under such circumstances as an index of the average temperature because the muscles are not represented in their proper proportionate value. However, these are the difficulties of dealing with the events of the first hour of cycling, and I propose to leave them on one side for the present, until in a more extended form of publication I can deal with the details of corrections for the temperature of the calorimeter and of the other measurements which add to form this picture. We shall therefore pass at once to a consideration of the events of the second hour.

The Second Hour of Cycling.

In each of the experiments represented by the data given subsequently the subject entered the calorimeter, and having been sealed in, remained seated until the balance of the surface of the calorimeter was well under control. He was then signalled to seat himself on the cycle, and at a second signal started to cycle at a fixed rate of 60 revolutions per minute. Outside the calorimeter window an incandescent lamp was made to glow 60 times a minute by the establishment of short-lasting contacts, one per second, by a seconds-clock, and it was the cyclist's duty to keep pace. His performance was watched by the cyclometer, and he was informed if the variations in his pace were noticeable. As a general rule, however, the rate was excellently maintained, and I owe many thanks to my long-suffering subjects, who pedalled away in this monotonous fashion in each case for a period of two hours.

The values of the electrical brake were originally arranged so as to separate the experiments into two groups. In one group the work done upon the cycle was to be maintained at a value of 13 kalories per hour; in the second group at a greater value, 43 kalories. These values were obtainable by the maintenance of the 60-per-minute revolution rate in each case and by sending in the one case a current of 1 ampère and in the other of

2·1 ampères through the electromagnets of the cycle, as was known from the results of calibration experiments most kindly performed for me in the Electrical Engineering Department of the University by Mr. Bissett, under the supervision of Mr. Crapper, to both of whom I am greatly indebted.

When a considerable number of such experiments had been performed with results all placed within certain limits of the scale of possible heat-production I was suddenly introduced to two simultaneous phenomena, (*a*) the ammeter connected with the circuit of the cycle brake, although mainly preserving an appearance of carrying the required currents, yet showed sudden intermissions during which the current evidently fell in value for short periods of time that seemed to be associated with some regular phase in the revolution of the cycle, and (*b*) that my experimental results now began to occupy new regions in the scale of possible heat-production. An examination of the electromagnets of the cycle-brake revealed a wire insecurely held within a soldered joint in such a fashion that the total resistance of the joint must have been very variable.

This fact is my quite sufficient excuse for omitting this particular group of experimental results. It also involved the complete rewinding of the electromagnet, since the fractured wire was buried by other coils, and therefore very inaccessible. I was thus involved in further consequences since I was obliged to recalibrate the cycle-brake within the laboratory. Two sets of calibration experiments were performed, (1) with 1 ampère, and (2) with 2·1 ampères in the altered brake. I now, however, found myself in each of these cases possessed of a more powerful brake than before, and rather than spend further time in discovering the particular values of current which would give precisely the same brake-power as formerly I proceeded to perform two new groups of experiments, in one group 19 kalories being the measure of the mechanical work performed per hour, and in the other case 56 kalories. I have therefore now to deal with four groups of experiments, and from the results it might perhaps appear that this is an advantage rather than a misfortune.

Before detailing these results I should make some mention of the fact that in addition to omitting a certain group of experiments previously referred to, there are some others which I have deliberately omitted from my list. In the first place, one of my subjects, a laboratory boy, Armstrong, promised to be of great advantage because of his smaller weight of 44 kgrm., thus occupying a different region of the scale of weights than most of my other subjects. He had, however, no experience of cycling, and was thus, in my opinion, most handicapped in the experiments with only a slight resistance in the brake. Given plenty of work to do on such a treadmill the best way of performing it is soon discovered and adhered to when found, but with the scanty work of the light brake many needless additional movements may be performed without much personal inconvenience. On this ground I have omitted his earlier experiments, which were of this kind. Now on similar grounds, namely, that excessive movements were seen and commented upon at the time, I have omitted two experiments with the lightest brake performed by two other individuals. In both cases the appearances were so marked that these subjects were requested to provide me with another opportunity, and were kind enough to grant this. One of these repetitions, however, fell unfortunately in the period of ambiguity of the brake, the other coincides with the time of the altered brake and is recorded there.

With these exceptions all the experiments of this type performed since October, 1912, are summarised in the data given. Those experiments which are quoted from an earlier date than this are selections satisfying the conditions adhered to this year during this whole series of experiments, and are taken from a time when I was experimenting with various revolution rates and brakes as well as with modifications in handling the calorimeter. As a matter of fact this process of selection has involved the exclusion of only two experiments from amongst those which might have been available; one because the cycling was not continued sufficiently into the second hour and the other because of a quite unusual difference between the temperature of the entering and leaving air and between the temperature of the laboratory and that of the calorimeter. In no case except that of Armstrong would the inclusion of these experiments have altered the general character of the results obtained.

Group A.—Experiments in which a maintained rate of 60 revolutions per minute would have involved the performance of mechanical work on the cycle at the rate of 13 kalories per hour (approximately 0·02 horse-power).

	Date.	Weight.*	Revolutions per minute.	Name.	Heat production, in kalories per hour.
	1912.	kgrm.			
I	Nov. 13	54·6	59·4	Bennet	160
II	„ 18	58·8	60·1	Ward	181
III	„ 21	66·7	60·1	Sharrard	209
IV	May 22	55·7	60·7	Briscoe	169
V	„ 25	61·8	59·7	Chapman	184
VI	„ 30	62·0	59·8	Duftey	186

Average heat production 182 kalories per hour.

Maximal aberrations from this average $\left\{\begin{array}{l}+12·4 \text{ per cent.} \\ -13·9 \quad „\end{array}\right.$

* All weights given were taken with the subjects stripped.

Group B.—Experiments in which a maintained rate of 60 revolutions per minute would have involved the performance of mechanical work on the cycle at the rate of 19 kalories per hour (approximately 0·03 horse-power).

	Date.	Weight.	Revolutions per minute.	Name.	Heat production, in kalories per hour.
	1913.	kgrm.			
	Jan. 28	54·6	60	Bennet	205
	„ 29	54·6	60	„	190
	Feb. 4	54·6	60	„	202
	„ 3	54·6	60	„	182
	„ 5	54·6	60	„	188
I	—	54·6	60	Average of Bennet	193
II	Feb. 17	62·1	59·8	Kemp	218
III	March 3	50·3	60	Gamm	197
IV	„ 4	60·5	60	Rae	212
V	„ 5	43·7	60	Armstrong	177

Average of I, II, III, IV, V, 199 kalories per hour.

Maximal aberrations $\begin{cases} +\ 9·5 \text{ per cent.} \\ -10·0 \quad „ \end{cases}$

Group C.—Experiments in which a maintained rate of 60 revolutions per minute would have involved the performance of mechanical work on the cycle at the rate of 43 kalories per hour (approximately 0·07 horse-power).

	Date.	Weight.	Revolutions per minute.	Name.	Heat production, in kalories per hour.
	1912.	kgrm.			
	Nov. 7	54·6	59·8	Bennet	278
	„ 11	54·6	59·6	„	280
	Dec. 5	54·6	60·0	„	283
I	—	54·6	59·8	Average of Bennet	280
II	Nov. 14	58·8	60·0	Ward	298
III	„ 15	66·7	60·0	Sharrard	324
IV	„ 25	59·9	60·1	Turnbull	299
V	Dec. 2	60·5	60·0	Rae	317
VI	„ 18	43·7	60·0	Armstrong	279
VII	May 20	55·7	60·6	Briscoe	285

Average of I, II, III, IV, V, VI, VII, 297 kalories per hour.

Maximal aberrations $\begin{cases} +9·1 \text{ per cent.} \\ -6·1 \quad „ \end{cases}$

Group D.—Experiments in which a maintained rate of 60 revolutions per minute would have involved the performance of mechanical work on the cycle at the rate of 56 kalories per hour (approximately 0·09 horse-power).

	Date.	Weight.	Revolutions per minute.	Name.	Heat production, in kalories per hour.
	1913.	kgrm.			
	Feb. 18	62·1	60·4	Kemp	354
	„ 26	62·1	60·2	„	345
I	—	62·1	60·3	Average of Kemp	350
	Jan. 27	54·6	59·7	Bennet	338
	„ 30	54·6	60·0	„	332
	„ 31	54·6	60·1	„	336
	Feb. 13	54·6	59·0	„	333
	„ 19	54·6	60·0	„	338
II	—	54·6	59·8	Average of Bennet	335
III	Feb. 20	60·5	60·4	Rae	347
IV	„ 21	60·4	60·5	Hill	345
V	„ 24	68·3	60·6	Sharrard	352
	„ 25	43·7	60·4	Armstrong	340
	„ 28	43·7	60·3	„	352
VI	—	43·7	60·4	Average of Armstrong	346

Average of I, II, III, IV, V, VI, heat-production of 346 kalories per hour.

Maximal aberrations $\begin{cases} +1\cdot7 \text{ per cent.} \\ -3\cdot2 \quad ,, \end{cases}$

Now, it is always of interest to study the relationship between such figures, and significant powers of the subjects' weights: that is to say, such powers as W the weight; or W$^{2/3}$, possessing some reference to the subject's extent of surface; or W$^{1/3}$, in which there always lies the possibility that it is W/W$^{2/3}$, or the subject's weight divided by his surface. That there is a very real interest in such a quest is well known in the special case of heat-production during rest, which bears a fairly close numerical relationship to the extent of the surface when other conditions remain the same. I have therefore divided the figures in these different groups by W$^{1/3}$, W$^{2/3}$, W, W$^{4/3}$, and W$^{5/3}$, thus obtaining, in each case, a set of figures with a certain average value, and give, in the table on p. 110, the maximal aberrations observed from this average value.

Maximal Aberrations, given as a percentage of the average result of dividing by various simple functions of W.

	$W^{1/3}$.	$W^{2/3}$.	W.	$W^{4/3}$.	$W^{5/3}$.
Group A	+ 9·5 −10·2	+ 7·6 − 5·8	± 3·3	± 2·5	+ 5·1 − 4·5
Group B	+ 4·4 − 2·7	± 3·6	+ 9·8 − 5·6	+16·8 −10·0	+20·7 −17·3
Group C	+ 5·5 − 4·9	+11·4 − 3·5	+22·0 − 7·0	+32·0 −12·4	+43·0 −18·4
Group D	+11·5 − 9·0	+19·8 − 9·0	+30·6 −14·9	+39·0 −19·7	+55·0 −25·0

Now, compare with the figures in this table the maximal aberrations from the average experimental result as given in the preceding tables.

Maximal Aberrations from the Mean Experimental Result.

Group A	12·4	and	−13·9
Group B	9·5	„	−10
Group C	9·1	„	− 6
Group D	1·7	„	− 3·2

It will be seen at once that the experiments of Group D gain nothing by any new process of numerical treatment. The experimental results are almost constant notwithstanding considerable differences in the weights, and therefore in various powers of the weights, of the subjects; and, indeed, if the observations were sufficient in number, it would be justifiable to write the general summary of the results obtained at this level of mechanical-work performance as

$$\text{Heat-production} = K_d.$$

If, on the other hand, we go to the other extreme and examine the results in Group A the original errors are as large as 13 per cent. of the mean experimental result, and it is seen that division of these results by almost any one of these functions of the weight brings them to a more common level, but that this is best done by dividing them by $W^{4/3}$, when the aberrations are only 2·5 per cent. from the average. Here, we might say that, at this much lower level of mechanical-work performance,

$$\text{Heat-production} = K_a W^{4/3}.$$

Treating the other groups in the same way we get the following summary of the experimental results :—

Group A.—Heat-production $= K_aW^{4/3}$
Group B.— „ $= K_bW^{2/3}$
Group C.— „ $= K_cW^{1/3}$
Group D.— „ $= K_d$

Clearly these results demonstrate the decreasing influence of the weight upon the heat-production as increasing values are given to the performance of mechanical work. To put the facts in simple terms, without attempting any further analysis, the weight becomes less and less of a handicap as the rate of work is increased, until at the final level reached in these experiments the burden of the day is the same for all. This does not say that it may be borne equally well by all. Small bodies embroiled in an equal heat-production are obviously at a disadvantage, since, although their surfaces and thus their means of heat loss are relatively large in proportion to their weights, yet they are actually smaller than the larger surfaces surrounding the greater masses. Thus higher temperatures might be thought of as impending in their case.

It will be of interest to continue these experiments, and that, too, at higher levels of work-performance. It would, for example, be well to determine whether this was the end or not of this process in the removal of the handicap of the weight. It would be remarkable if at some higher level of work-performance the weight should be developed into a positive advantage, and the relationship be capable of expression as follows:—

$$\text{Heat-production} = KW^{-n}.$$

Leaving this, however, for the present alone, it is legitimate to inquire into the possible causation of these results as they stand. Thus, when dealing with the nature of the process responsible for the appearance of the weight in these results, it will be well at once to focus attention on this query: Is any importance to be assigned to the observation at the lowest level of work-performance, in which the weight made its appearance in the greater dignity of the form of $W^{4/3}$? For, if not, it would be simple to consider that all of these expressions are variations from the well known expression for the heat-production of rest (heat-production $= KW^{2/3}$), in which the importance of the surface, $W^{2/3}$, is gradually removed with the increasing elimination of the activity of that special nervous mechanism that regulates heat-production when heat-loss is the dominant circumstance. But, if, on the other hand, attention must be paid to it—and why not?—then some consideration must be given to some influence of the weight or of the mass of the body other than as a value which determines the extent of its surface. This may conceivably be dealt with under two headings.

Thus, on the one hand, it may be thought that the weight, *per se*, makes its appearance in these expressions because the weight of the limbs that are moved is at first a large fraction of the whole sum of mechanical work which is performed. Or, on the other hand, it might be considered that W enters in some less direct fashion as a representative of mass rather than of weight, since it is clear that the value "heat-production/mass" must to some degree determine the temperature of the musculature, and further, as is well known to be true, the heat-production would be expected to vary with this temperature, as well as with the mechanical work which is performed.

On either of these assumptions as to the importance of W, it is conceivable that at first the expression for the heat-production of rest was complicated in such a fashion that

$$\text{Production/Surface} \quad \text{became} \quad \text{Production}/(\text{W} \times \text{Surface});$$

that is to say, that the denominator might change from $W^{2/3}$ to $W^{5/3}$. Now we have only to consider that the withdrawal of the dominating influence of the surface, the removal of one kind of automatic nervous control, passes on further than to mere elimination until the surface becomes now the humble agent of the heat-production under the influence of a reversed nervous control. This is, indeed, what actually happens, but is it not the case, this being so, that now the heat-production will tend to vary inversely as the surface?

$$\frac{\text{Heat-production}}{\text{W} \times \text{Surface}} \quad \text{now becomes} \quad \frac{\text{Heat-production} \times \text{Surface}}{\text{W}},$$

and the denominator changes from $W^{5/3}$ to $W^{1/3}$.

Now, finally, we come to consider the state of affairs represented by Group D, where, apparently, both weight and surface have dropped out of account. Here it is open to us to consider that the surface has now become a cubic quantity with the same dimensions as the weight, and that they are thus both cut out from the expression for the heat-production. Nor is this so absurd as might at first appear, since the process of sweating introduces a further quantity, by which the extent of the surface is multiplied, and might very well be regarded as a third dimension. Or else we revert to the position that the weight of the limbs becomes less and less important as the amount of externally useful mechanical work rises in value, and that in this way there is a tendency to minimise the value of W in the denominator. If this is so, then it is to be anticipated that a further continuance of these experiments will lead to the further observation that, at a still higher level of work-performance, the heat-production will be found equal to KW^{-n}, and the weight then have an appearance of being a positive advantage, since there is then no obvious reason why the surface should disappear from the numerator.

The Formation of the Anthocyan Pigments of Plants.—Part VI.

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In the previous communications of this series we have recorded the results of observations on the oxydases and chromogens concerned with the production of anthocyan pigments. The study of pigment formation is continued in the present communication, the sections of which deal with the following subjects:—

1. A pigment-producing glucoside of the wallflower (*Cheiranthus cheiri*).
2. The formation of pigment-producing substances from glucosides.
3. The biochemistry of Mendelian colour characters.

Section 1.—*A Pigment-producing Glucoside of the Wallflower.*

It is customary to divide the sap-pigments of plants into two series, the red, purple, and blue anthocyan pigments, and the yellow xanthein pigments. Miss Wheldale[*] has, however, suggested, on genetical grounds, that the anthocyan and xanthein pigments are related with one another. This author points out that most plants contain colourless or pale yellow substances which give a canary yellow colour with ammonia. When heated with dilute acid they assume a deep yellow colour and reduce Fehling's solution. Hence, they are to be regarded as glucosides.[†]

Miss Wheldale suggests that anthocyan is a compound of such a glucoside-like body with a "reddening" substance. In the absence of the latter and with the loss of a further substance (the sugar ?), the glucoside gives rise to a yellow xanthein pigment.

More recently,[‡] Miss Wheldale states that the yellow pigments are largely present as glucosides, of which some, or possibly all, the hydroxyl groups are replaced by sugar. Specific hydrolysis may act on hydroxyl groups in certain positions, and when these groups are free from sugar, oxidation, and possibly condensation, may take place at these points. The residual hydroxyl groups in the anthocyan molecule would probably be replaced by sugar, and hence the anthocyans would occur as glucosides.

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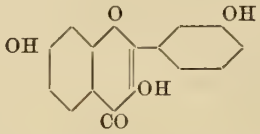
[*] 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 44.

[†] Compare Czapek, 'Biochem. d. Pflanzen,' vol. 1, p. 177.

[‡] 'Biochem. Journ.,' 1913, vol. 7, p. 87.

It is to be remarked that this hypothesis postulates a larger number of glucose residues in the molecule of the yellow pigment than is the case in the varieties which have been studied. In none of these are more than two sugar residues present. Moreover, even in such cases, the sugar residues are not attached to different hydroxyl groups, but are united to one another to form a disaccharide. Further, the experience gained with amygdalin* suggests that such complex glucosides are only broken down by enzymes in one way, and hence, if this be the case, it is not probable that different enzymes act on the glucosides in such a manner as to set free different groups.

The yellow pigments are regarded by Miss Wheldale as belonging to the flavone or xanthone classes, and in this connection it may be pointed out that the constitution of the hydroxyflavone glucosides offers great possibilities of variation. Thus the sugar residue, which may be either glucose or rhamnose, is joined by an hydroxyl group to the flavone. The hydroxyl group may be attached to carbon in the oxygen ring or substituted in either of the phenol groups.



Such differences of constitution will correspond with differences in the properties of the several glucosides, and these different properties will be manifested particularly when the glucosides are acted on by enzymes or hydrolysed by acids.

A further possibility of the derivation of varied products from these glucosides follows from A. G. Perkin's observations (*vide infra*) to the effect that the glucosides of this class occur commonly in plants not singly but in association. In a valuable series of memoirs dating from 1895, A. G. Perkin has described a number of natural hydroxyflavone glucosides, and at the present time the structure of at least 12 of these bodies is known. The researches of Perkin have been supplemented by Kostanecki's synthetical work on the hydroxyflavone group.

The outstanding fact revealed by Perkin's investigations is that, of the yellow pigments derived from many and diverse varieties of plants, all, without exception, are hydroxyflavone derivatives; indeed, with this evidence before us, it is not too much to assume that all the soluble yellow pigments of plants belong to this class. The observations described in this section have reference to the soluble sap pigments of the wallflower (*Cheiranthus cheiri*). Of these pigments, A. G. Perkin* has already investigated the hydroxyflavones present in the deep yellow flower of the variety of wallflower known as "Cloth of Gold." He has shown that when the extract of sap pigment is hydrolysed by boiling with dilute sulphuric acid an olive yellow precipitate of the pigment separates on cooling. The precipitate is a mixture of quercetin

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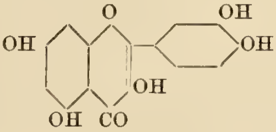
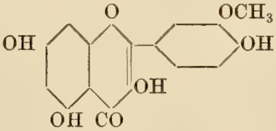
* Compare Armstrong, 'The Simple Carbohydrates,' 1912.



and its monomethyl ester iso-rhamnetin



Of these substances quercetin is distributed very widely in plants, but iso-rhamnetin has been met with only in the Indian dye, asbarg (from *Delphinium zalil*).†

The general characters and distribution of the pigments of the flowers of *Cheiranthus cheiri* are as follows:—

The petals contain both sap and plastid pigments. In the deep yellow varieties the colour is due to a plastid pigment which accompanies and masks a pale yellow (cream) sap pigment. Purple flowers contain a purple (anthocyan) pigment, together with a little plastid yellow. The same pigments occur in the brown wallflower, which owes its colour to the large amount of yellow which occurs, together with purple. Red flowers contain anthocyanin, together with xanthein, the yellow of which masks to some extent the blue constituent of the anthocyan pigment. In certain primrose-yellow varieties the colour changes with the age of the flower. The buds and newly opened blossoms are of a rich yellow, but as the flower grows older the yellow fades gradually till the petals are cream or almost white. The full yellow is due to the plastid pigment which occurs together with the pale primrose-coloured xanthein in the buds of young flowers. The plastid pigment disappears as the age of the flower increases, and the pale colour of the full blown flower is due to the persistence of the soluble sap or xanthein pigment.

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* 'Chem. Soc. Trans.,' 1896, p. 1566.

† Perkin and Pilgrim, 'Chem. Soc. Trans.,' 1898, p. 267.

The waning of the yellow plastid pigment coincides with the disappearance from the petals of an inhibitor of oxydase. Thus, if petals of various stages are tested by means of benzidine and hydrogen peroxide, only the mature cream-white petals, from which the yellow pigment has disappeared, give an oxydase reaction. If, however, the petals are first treated for five minutes with absolute alcohol, and then with benzidine and hydrogen peroxide, they give a good oxydase reaction.

Inasmuch as we have shown already (Part IV) that absolute alcohol serves to remove the inhibitor of oxydase which occurs in the flowers of dominant white *Primula sinensis*, we conclude that there exists in this "primrose" strain of wallflower an oxydase inhibitor which is closely bound up with the yellow plastid pigment. As the latter is destroyed so is the former, and after their disappearance the oxydase contained in the cells in which they were present may be demonstrated by means of the ordinary oxydase reagents.

These observations, together with the fact that red and purple anthocyan only make their appearance in cells in which the plastids are degenerating, may, perhaps, offer a clue to the significance of the curious phenomenon of striping which is so common in wallflowers. In striped wallflowers the red or brown colour is broken by wider or narrower yellow bands. We believe that the phenomenon is to be interpreted in terms, first of the persistence of the plastids and of the inhibitor of oxydase, and, second, of failure of supply of chromogen.

That the former of these factors is concerned in the phenomenon is probable from the foregoing observations, and that lack of chromogen is also concerned is rendered probable by observations which we have made on the striped flowers of Honesty (*Lunaria annua*).

The garden varieties of this plant bear flowers showing all degrees of striping. In some the petals are self (uniformly) coloured; in others there is a small colourless area at the junction of limb and claw, and in others this white area may extend so far as to leave but a narrow border of magenta at the edge of the petal. The white regions of the flower are well supplied with peroxydase, and therefore their lack of colour is to be ascribed to lack of chromogen. That this is actually the case may be demonstrated by removing all the open flowers and some of the buds from a head of flowers. As a result of thus reducing the number of flowers of the inflorescence, those that are left assume in what should be the white region of the flowers a dark magenta colour, so dark as to look like a deep magenta splash on a pale ground. Into the physiological interpretation of this effect of the operation we need not enter now, but we may take it as demonstrating that streaking or flaking of flowers is due, in some cases, to local inhibition of oxydase, and in others to a local defect of chromogen.

That the pale yellow or cream xanthein sap pigment of the wallflower is in some way related with the red and purple anthocyans is rendered probable from a study of the curious behaviour of a hybrid wallflower *Cheiranthus kewensis*, the issue of a cross between *Cheiranthus cheiri* and *C. mutabilis.* When the flowers of *C. kewensis* open they are of a pale yellow or cream colour, and of that colour they remain for a long time. Gradually, however, a faint reddish hue steals over them, deepens, and finally replaces the original colour. Experiments now to be described make it probable that what is witnessed here is a gradual formation of red anthocyan at the expense of the cream-yellow xanthein pigment.

If petals of the "primrose" race of the common wallflower, *C. cheiri*, or the soluble yellow extract therefrom be heated in aqueous alcoholic solution with a little concentrated hydrochloric acid and zinc dust a red pigment is formed. In the first phase of the reaction the xanthein glucoside is hydrolysed to a reducing sugar and to a yellow compound which is insoluble in water. For example, a solution of the pigment was divided into two parts, and the reducing power of the solutions was determined by the Bertrand method, in the one before and in the other after hydrolysis. The results were:—

Before hydrolysis = sugar equivalent to 6·5 c.c. of permanganate.
After „ „ „ 13·5 „ „

The glucoside is also hydrolysed slowly by the emulsin of almonds. After the yellow insoluble compound produced during hydrolysis has been dissolved in 50-per-cent. alcohol and reduced by zinc dust and an acid it undergoes reoxidation to an intense red pigment. The red pigment passes through green to yellow on the cautious addition of alkali. It is reduced to a colourless state by zinc dust and acetic acid or by zinc dust and ammonium chloride, the colour recovering on oxidation.

The solution of hydrolysed pigment separated by filtration from the yellow product gives no red colour when reduced. Thus we have chemical evidence that the yellow glucoside of the wallflower undergoes hydrolysis, and that its product is converted by reduction and oxidation into a red pigment. Since reduction and oxidation may take place in any plant cell we may infer that the pale yellow wallflower owes its lack of red or purple anthocyan to the absence of the agent for glucoside hydrolysis, and it may be predicted that the pale colour of the primrose (yellow-cream) race of wallflower is recessive to the anthocyan colour of red or purple races.

We have obtained by the means described already red pigments from the yellow and colourless flowers of many other plants, *e.g.*, yellow daffodil, yellow crocus, cream polyanthus, and chinese primrose. As a rule the colour is located at first especially in the veins, but it appears subsequently in the whole petal. The case of the daffodil is of particular interest, inasmuch as plant breeders have been at work for many years endeavouring to produce a red daffodil. This work has met already with some considerable success, and it may be predicted on biochemical grounds that the object will be achieved completely in the near future.

In the case of some flowers, *e.g.*, polyanthus, reduction by means of zinc dust is not necessary, the red coloration appearing after heating with acid and subsequent oxidation.

We have used the same reagents with the flowers of dominant and recessive white *Primula sinensis*, and find that, as is to be expected on theoretical grounds, dominant whites yield the red colour, and recessive whites, if they yield it at all, do so to an extremely slight extent. Thus we have biochemical evidence supplementary to that derived from plant-breeding experiments that dominant whites possess and recessive whites lack the prochromogen from which anthocyan pigments are derived.

We have described already (Part IV) the recovery of colour which takes place when a decolorised petal of the stock (*Matthiola incana*) is immersed in water, and we have stated our reasons for regarding the recovery of natural colour as the result of a process of oxidation. Subsequent experiments confirm this view.

Thus, by treating an aqueous solution of the purple pigment of stocks with zinc dust and acetic acid in the cold, the pigment becomes first pink, and is reduced subsequently to a colourless state. The colour returns rapidly on exposure to air, and still more quickly when the solution is warmed or treated with a drop of hydrogen peroxide. In each case the original purple is recovered. If zinc dust and ammonium chloride be used, reduction to a colourless state also takes place, but subsequent oxidation leads to the formation of first a pale green and then a blue colour.

Section 2.—*The Formation of Pigment-producing Substances from Glucosides.*

The present state of our knowledge does not allow of a perfectly satisfactory classification of the pigments which commonly occur in the flowers of plants. Nevertheless, these pigments may be grouped provisionally according to their mode of origin and chemical composition.

Classified according to their modes of origin, the flower pigments fall into two groups, those which are derived from the plastids, and those which are formed independently of the plastids in special vacuoles of the cytoplasm. The former are called plast pigments and the latter sap pigments. In the mature cell the sap pigments occur in solution with the general cell sap, and hence they are referred to sometimes as the soluble sap pigments. They occur in two series, the soluble yellow pigments (see p. 115) and the so-called anthocyan pigments. Evidence has been given already (p. 117) to show that the soluble yellow pigments are to be regarded as flavone derivatives, and that they are related with the red and blue anthocyan pigments.

Of the plast pigments, two series also occur. One comprises the yellow and red carrotene pigments, together with the oxidised derivatives of the latter, the xanthophylls. The other series includes the chlorophyll pigments. Of these pigments, carrotene (a hydrocarbon) contains C and H, xanthophyll, the soluble yellow sap pigment, and the anthocyans contain C, H, O, and chlorophyll, like hæmoglobin and the animal melanins, contains N in addition to these elements.

It is known that the melanins of animal tissues are produced by the action of tyrosinase on chromogens. We show now that pigments containing nitrogen are formed readily by the action of plant enzymes on the glucosides which occur in plants. Hence it is probable that the formation of such nitrogen-containing pigments occurs normally in the living plant.

Two recently published researches of Chodat have important bearings on the mode of pigment formation in plants. In the one research, Chodat* proves that, when a vegetable oxydase acts on glycine, carbon dioxide, formaldehyde, and ammonia are produced. In the other, he demonstrates that, when *p*-cresol is oxidised by oxydase in the presence of an amino-compound, a series of coloured substances is produced, the colours of which depend on the nature of the amino-compound.

We have confirmed the accuracy of Chodat's conclusions and have applied them to an investigation of the behaviour of certain glucosides, arbutin, methyl arbutin, and others, when acted on by plant enzymes.

Arbutin and methyl arbutin are distributed widely among species of the genus Pyrus and also among the members of the Ericaceæ and certain other natural orders. When hydrolysed by emulsin, arbutin and methyl arbutin yield respectively quinol and its mono-methyl derivative. Inasmuch as emulsin usually contains a small quantity of oxydase, hydrolysis is accompanied by a darkening of the solution owing to the oxidation of the quinol. It has been suggested by Bourquelot and Fichtenholz that the natural blackening which takes place in the fallen leaves of many species of Pyrus is due to the oxidation of quinol.

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* 'Arch. Sci. Phys. Nat.,' 1913, vol. 35, p. 140.

We now find that if a mixture of arbutin and glycine be hydrolysed by emulsin and then oxidised by means of the oxydase present in an aqueous solution of bran, an intense red coloration is produced. The red substance is evidently derived from the interaction of *p*-quinone with glycine, or its oxidation products formaldehyde and ammonia. A similar red colour is obtained when, instead of glycine, alanine, leucine, phenylalanine, tyrosine, or asparagine is used. The colour is also produced when arbutin is incubated at 37° with a little ground sweet almond and a drop of hydrogen peroxide. The almond contains emulsin, peroxydase and amino-acids, so that it allows of the whole series of changes.

The changes may be represented schematically as follows:—

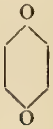
$$\text{Arbutin} + \text{water} \xrightarrow{\text{Emulsin.}} \text{quinol} + \text{glucose.}$$

$$\text{Quinol} + \text{oxygen} \xrightarrow{\text{Oxydase.}} \text{quinone.}$$

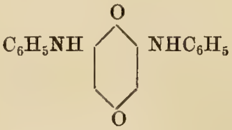
$$\text{Glycine} + \text{oxygen} \xrightarrow{\text{Oxydase.}} \text{formaldehyde, ammonia, and carbon dioxide.}$$

$$\text{Quinol} + \text{quinone} + NH_3 = \text{red compound.}$$

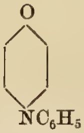
The production of a red colour from quinone and amino-acids was first observed by Wurster* and later by Raciborski,† but neither author offers any explanation of the phenomenon. The reaction has been studied recently by E. A. Cooper,‡ who holds that the colour results from the interaction of the $-NH_2$ groups of the amino-acid and the oxygen atoms of the quinone, and that the reaction is similar to that which takes place between an amine, such as aniline, and quinone. In the latter case the aniline residues may enter the ring in the 1, 3 positions, or, in addition, form an anil or dianil by replacing the quinone oxygen.



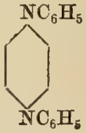
Quinone.



Dianilinoquinone.



Quinone anil.



Quinone dianil.

The abnormal behaviour of the amino-acids, under conditions when other compounds containing a primary amino-group form condensation products, was shown originally by Strecker.§ He proved that alloxan oxidises alanine to carbon dioxide, ammonia, and acetaldehyde. Hurtley and Wooton,* wh[illegible] have made a full study of the reaction, find that dimethylalloxan behaves in a similar manner. W. Traube† has found that benzoquinone and isatin have similar oxidising properties.

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* 'Chem. Zentr.,' 1889, vol. 1, p. 392.

† 'Chem. Zentr.,' 1907, vol. 1, p. 1595.

‡ 'Biochem. Journ.,' 1913, vol. 7, p. 186.

§ 'Annalen,' 1863, vol. 123, p. 363.

Glycine ethyl ester, $NH_2.CH_2.CO.OC_2H_5$,‡ behaves quite differently. With quinone in alcoholic solution it forms the di-ethyl ester of diglycinoquinone, together with hydroquinone; the amino-acid is not decomposed, since the ester group shields the carboxyl group from attack.

It is improbable, therefore, in view of these observations that the condensation between quinone and glycine takes place in the manner suggested by Cooper.

Ammonia by itself gives a brown coloration with quinone, but if formaldehyde be added the brown colour is converted into red. No coloration is given on mixing formaldehyde with quinone, but on the cautious addition of ammonia a red coloration is produced. The colour is very similar to that given by quinone and glycine; this last mixture gives the same reddish-brown shades when a slight excess of ammonia is added. Similarly, quinone gives no colour with benzaldehyde alone, but a red colour is produced on the addition of ammonia, the colour resembling the red obtained from phenylalanine and quinone. Salicylic aldehyde, quinone, and ammonia give rise at first to a red coloration and then to an insoluble brown substance.

Quinone forms a red coloration alike with glycine, alanine, leucine, tyrosine, phenylalanine, or asparagine. The colours are very similar to, not identical with, one another, and they are formed at much the same rate. The red is reduced immediately by zinc dust and acetic acid, and a colourless solution is obtained (see Section 1). The colour returns slowly on standing, more quickly on warming, and immediately on the addition of a drop of hydrogen peroxide.

Quinol is converted by an oxydase in presence of glycine into a red pigment; of its ethers dimethylquinol gives no coloration, and methylquinol a faint pink only, which is, perhaps, due to impurity or to its oxidation to quinone. Methyl arbutin should not, therefore, be capable of giving rise to this red pigment.

When the oxidation of arbutin at 37° is prolonged, action continues past the red stage. The solution becomes a chestnut brown, a brownish black precipitate is deposited, a little tarry matter appears on the surface, and a marked aromatic odour suggesting the smell of prunes is imparted to the liquid.

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* 'Chem. Soc. Trans.,' 1911, vol. 99, p. 288.

† 'Ber.,' 1911, vol. 44, p. 3145.

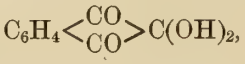
‡ E. Fischer and Schrader, 'Ber.,' 1910, vol. 43, p. 525.

This conversion of a glucoside into substances which simulate both the colour and odour of ripe fruit is noteworthy, and suggests that in the natural process of ripening of fruits, not only are glucosides hydrolysed and certain of their products oxidised by oxydases, but also that amino-compounds, so common in plants, intervene in the reaction and determine the nature of the end products.

The reaction between quinone and the products of oxidation of the amino-acids is obviously very complicated, and we therefore content ourselves with a reference to two somewhat similar cases which have been emphasised as of biological importance :—

1. Strecker and Hurtley and Wooton have shown that by the interaction of glycine and alloxan, $\mathrm{CO}{<}\substack{\mathrm{NH.CO}\\ \mathrm{NH.CO}}{>}\mathrm{C(OH)_2}$, at the ordinary temperature, the amino-acid is oxidised to formaldehyde, carbon dioxide, and ammonia, whereas the alloxan is in part reduced to dialuric acid. The unchanged alloxan and dialuric acid combine to produce alloxantin, which with the ammonia forms murexide (ammonium purpurate). This substance has a characteristic purple colour. On warming the mixture, secondary changes take place with the formation of other coloured products, and in the case of tyrosine Hurtley and Wooton note that a " flowery " odour is produced.

2. Ruhemann has shown that triketohydrindene hydrate,



$$\mathrm{C_6H_4}{<}\substack{\mathrm{CO}\\ \mathrm{CO}}{>}\mathrm{C(OH)_2},$$

which gives a deep blue coloration with amino-acids, behaves like alloxan. The hydrindene is now regarded as a valuable reagent for amino-acids, and it has already been of service in investigating problems of animal physiology. In this case also the amino-acid is oxidised to carbon dioxide, ammonia, and aldehyde, and the triketohydrindene is reduced. Hydrindanthin is produced and interacts with ammonia to form the blue ammonium salt of diketohydrindylidene-diketohydrindamine, $\mathrm{C_6H_4}{<}\substack{\mathrm{CO}\\ \mathrm{CO}}{>}\mathrm{CH.N{:}C}{<}\substack{\mathrm{CO}\\ \mathrm{CO}}{>}\mathrm{C_6H_4}$, which is an analogue of murexide.

It is probable that similar changes take place in the case of quinone and amino-acids. Starting from arbutin, where the hydroquinone which is formed is oxidised in part to quinone, it is the ammonia from the amino-acid which is the essential factor in producing the red colour. The aldehyde derived from the amino-acid plays only an accessory part, for the reduced quinone is already present. In fact, the red is instantly produced when ammonia is added to the oxidised mixture. The addition of formaldehyde to hydrolysed and oxidised arbutin has no effect in producing colour, and this is in agreement with the fact that the red colour is produced no matter what amino-acid be used. A series of similar experiments was made differing only in that different amino-acids in equivalent quantity were added in the several cases. The colours produced were compared with one another by means of the tintometer in order to obtain a rough indication of their relations with one another. The same series was again examined a few days later, when the solutions had become darker.

Amino-compound used.	Colour produced.
Glycine	value = 8 red + 13 yellow.
Alanine	" = 8 " + 11 "
Leucine	" = 7·5 " + 10 "
Phenylalanine	" = 5 " + 6 "
Tyrosine	" = 5·5 " + 6 "

The three aliphatic acids give similar colours, the two aromatic acids yield a somewhat different shade. Hence these pigments differ essentially from those obtained by Chodat from *p*-cresol, inasmuch as the colours of the latter depend on the nature of the amino-acid. Whatever be the explanation, the formation of pigment from arbutin and protein degradation-products is one which may well be of natural occurrence. In passing, it may be observed that quinone, like alloxan and triketohydrindene, may prove to be of use in the diagnosis of amino-compounds.

Substituted quinones such as 1:4-xyloquinone or 1:4-thymoquinone resemble quinone in giving a colour reaction with glycine on warming in aqueous-alcoholic solution; but in the case of these substances the reaction takes place much more slowly. Xyloquinone give rises to a claret red, thymoquinone to a tawny or brown red. There is apparently a difficulty in reducing the quinones, as neither of them gives a colour reaction with formaldehyde and ammonia.

We are investigating the behaviour of other glucosides and find that salicin, the glucoside of the willow and many other plants, gives an orange, passing to an orange-red, coloration when hydrolysed by emulsin and oxidised by an oxydase in presence of an amino-acid. Similar colours are obtained with glycine and with phenylalanine, the tintometer reading in a half-inch cell being in each instance 4·5 red + 1·8 yellow. Salicin incubated with ground sweet almond and a few drops of hydrogen peroxide gives a similar colour reaction.

Phloridzin, the glucoside present in the roots of many rosaceous trees, is composed of glucose and phloretin, a condensation product of *p*-hydroxy-hydratropic acid and phloroglucinol. When hydrolysed by emulsin in presence of glycine it is converted into a yellow substance which becomes orange and finally orange red. A red insoluble deposit, which separates out, forms an orange-red solution in alcohol. In the tintometer we find for a $\frac{1}{8}$-inch cell: alcoholic extract, 3·5 red + 1·5 yellow; aqueous solution, 2·5 red + 5 yellow.

Finally, æsculin (from the horse-chestnut) gives a yellow precipitate, and aucubin (from the red berries of *Aucuba japonica*), a black precipitate under the conditions described.

The property of colour formation from a glucoside and an amino-acid seems to be a very general one, though we are unable to say whether the mechanism is in each case the same as we have postulated for arbutin, namely, oxidation of the phenol to a quinone, formation of a quinohydrone and interaction of this with ammonia to form a coloured salt.

In any case, Chodat's discovery of the resolution of amino-acid into formaldehyde and ammonia is obviously of fundamental importance. The ammonia may serve to provide the alkaline conditions so favourable for oxidation and it may react directly to form amino-compounds. The formaldehyde may take part in all manner of condensations leading to the production of complex substances.

Section 3.—*The Biochemistry of Mendelian Colour Characters.*

Of the various artificial chromogens which serve to determine the presence of oxydase in flowers, benzidine behaves most like the natural chromogens. For example, most artificial chromogens, α-naphthol, guaiacol, etc., serve well enough to indicate the presence of oxydase in the vascular tissues (veins), but they do not react as a rule with the oxydase contained in the epidermal cells, whereas benzidine gives uniformly good reactions with both epidermal and bundle oxydase (see Part I). Again, just as the reducing agents present in petals may reduce the anthocyan pigments to a colourless state, so these same agents reduce and decolorise the blue oxidation product of the interaction of plant oxydase and benzidine.

Inasmuch as benzidine has proved to be of considerable value for the investigation of plant oxydases, it may be useful to preface this section with a brief account of what is known of the oxidation products of benzidine, which are of an unusually complex character.

Willstätter and Kalb* have shown that the first oxidation product of benzidine ($NH_2C_6H_4.C_6H_4NH_2$) is probably the reddish-brown diphenoquinone di-imine $NH:C_6H_4:C_6H_4:NH$. On further more drastic oxidation, two molecules of this substance unite to form the yellowish-red diaminoazodiphenyl $NH_2.C_6H_4.C_6H_4.N:N.C_6H_4.C_6H_4.NH_2$. The blue- and violet-browns, so characteristic of the action of oxydases on benzidine, are due to complex, partially or *meri*-quinonoid salts of diphenoquinone di-imine with benzidine itself:—

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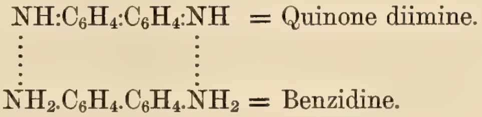
* 'Ber.,' 1905, vol. 38, p. 1232.



$$NH:C_6H_4:C_6H_4:NH = \text{Quinone diimine.}$$

$$NH_2.C_6H_4.C_6H_4.NH_2 = \text{Benzidine.}$$

The molecules are united through the partial valencies of the nitrogen atom. These compounds are *meri*-quinonoid, in that the quinone di-imine may be combined with several molecules of benzidine. For example, Willstätter and Piccard* describe a blue compound of the di-imine with four molecules of benzidine, and a brownish-violet compound with three molecules of the amine. Upon reduction, such *meri*-quinonoid compounds are converted into benzidine, whilst oxidation transforms them gradually into the quinone di-imine, as more and more of the benzidine is oxidised.

For the investigation of plant oxydase, and of inhibitors of oxydase, we find that it is convenient to use benzidine in two forms, viz., a ½-per-cent. solution in 50-per-cent. alcohol, and a saturated solution in 1–2 per cent. of sodium chloride.† When rapidity of action is required, the latter solution is employed, but, when inhibition is under investigation, the alcoholic solution should be used side by side with the sodium chloride solution. In illustration of the rapidity of action of the sodium chloride benzidine solution, it may be mentioned that, if young seedlings of maize, etc., or mature roots of water plants such as *Hydrocharis morsus-ranæ* (frog bit) be immersed for a few minutes in this solution, the subsequent addition of a few drops of hydrogen peroxide causes almost instantaneously a bright blue coloration of their root-hair regions.

Again, if flowers known to contain an inhibitor of oxydase be treated with some agent, for example, absolute alcohol, which is known to remove the inhibitor (see Part IV), they fail to react with the alcoholic benzidine solution until the whole or greater part of the inhibitor has been removed, whereas such flower sgive a definite reaction with sodium chloride benzidine, even though the inhibitor has been only in part removed.

The subject with which we deal in this section is that of the cause of the range of flower-colour which occurs within a species. In illustration of the nature of this problem we may mention the facts known in the case of the flowers of the chinese primrose (*Primula sinensis*). In addition to white-flowered races (dominant and recessive whites), the horticultural varieties of this species comprise races with blue, red, and magenta flowers, and our purpose is to put forward a biochemical hypothesis to account for the production of these distinct colours and for the genetical relations which obtain between them.

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* 'Ber.,' 1908, vol. 41, pp. 1458, 3245.

† *Cf.* Madelung, 'Zeitsch. physiol. Chem.,' 1911, vol. 71, p. 204.

It has been suggested by Miss Wheldale* that each of the chief colours of such a series is determined by a special oxydase, but neither general considerations nor such observations as we have been able to make lend support to this view.

It is true that the flowers of different varieties of *P. sinensis* contain different amounts of oxydase, but we find no constant relation between amount of oxydase and type of coloration. Moreover, the recent researches of Bach† point definitely away from the hypothesis that oxydases are specific.

If hypothesis of specific oxydases be rejected, we must ascribe specific coloration either to the action of an oxydase on different chromogens or to the interaction, with a chromogen or an oxydase, of specific substances which modify decisively the colour produced in the course of the reaction.

Any discussion of these alternatives must take into account the observations of A. G. Perkin, that the hydroxyflavone glucosides of plants occur, as a rule, not singly but in groups. There is some ground for regarding these glucosides as constituting the prochromogens from which the anthocyan chromogens are derived, and it is therefore a matter of great significance to the student of genetics that the plant is, as it were, offered a choice of several pigment-forming materials on which its hydrolysing and oxidising enzymes may act.

Pending fuller investigation of the possibility that the colour of a variety may be determined by a selective action on one of a group of allied glucosides, we are inclined to adopt the latter of the two alternatives, and to suggest that the serial colours of flowers are due each to the intervention of specific substances in the reaction of oxydase on chromogen.

This hypothesis is rendered plausible by the following observations, first on the colours produced when a mixture of phenols is treated with oxydase and second, on the behaviour of our artificial chromogen benzidine when acted on by oxydase in the presence of various phenols.

When a mixture of phenols is treated with a plant oxydase a competition for oxygen ensues. For example, if oxydase be caused to act on guaiacol until the red colour is produced, the addition of other phenols brings about a more or less quick change of colour. Thus α-naphthol converts the red into mauve, and the ultimate colour which is produced is of a far deeper tint than that which arises when α-naphthol and oxydase interact with one another. If *p*-cresol be added to the red solution produced by the action of oxydase on guaiacol a brown colour appears. Saligenin, on the other hand, does not modify the normal deep red colour given by guaiacol and oxydase.

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* 'Prog. Rei Bot.,' 1910, p. 469.

† 'Arch. Sci. Phys. Nat.,' June, 1912, vol. 33.

Search in chemical literature brings to light a few records of similar observations. Thus Schoenbein, in 1856,* observes that guaiacum blue oxidises other oxidisable substances, and in doing so becomes reduced and decolorised. Kastle and Porch† find that the oxidation of *p*-phenylene diamine, guaiacum, and phenolphthalein, by means of an oxydase, is accelerated greatly by phenol, the cresols and β-naphthol. They recognise that these accelerators act probably as auxiliary oxygen carriers, and that they are themselves more or less completely oxidised in the process. Miss Wheldale‡ suggests that oxidised catechol acts as a peroxide.

p-Phenylene diamine (*p*-diamino-phenyl, $NH_2C_6H_4NH_2$) exhibits a somewhat different behaviour. Together with α-naphthol it constitutes the indophenol reaction for oxydase, which reaction is used largely by animal physiologists. In it the oxidised amine and phenol are coupled to an indophenol.

We find that phenylene diamine gives much the same violet-blue coloration with all phenols, including methyl quinol; but that benzidine and oxydase give with each phenol a distinct colour, similar to that produced when the phenol in question is oxidised by oxydase. It would appear, therefore, that nothing analogous with the indophenol reaction takes place when benzidine and phenols are acted on by oxydase.

It is to be noted that *p*-phenylene diamine is oxidised by atmospheric oxygen to a garnet red (tetra-aminodiphenyl-*p*-azophenylene), whereas it gives a dark brown product when oxidised by oxydase. The indophenol reagent is ill-adapted for the localisation of plant oxydases because of the readiness with which it oxidises spontaneously. Petals of recessive white *Primula sinensis* give a general purple reaction with it, and a brown with phenylene diamine, but solutions of these reagents become strongly coloured even without the addition of hydrogen peroxide, whereas this is not the case with benzidine and the other reagents which we use for the investigation of plant oxydases.

We have not used *as*-dimethyl-*p*-phenylene diamine ($NH_2C_6H_4NMe_2$), which substance is oxidised readily to quinone imine, the salts of which form red meri-quinoid compounds with the unchanged diamine—the so-called Wurster salts.

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* 'Journ. Prakt. Chem.,' vol. 57, p. 496.

† 'Journ. Biol. Chem.,' 1908, vol. 4, p. 301.

‡ 'Roy. Soc. Proc.,' 1911, B, vol. 84.

We have noted already that when hydroquinone is added to a mixture of benzidine and oxydase in which the blue colour has been allowed to develop the colour is discharged. It is not until all the hydroquinone has been oxidised that the blue colour begins to return, the limiting factor being the amount of hydrogen peroxide present. Most other phenols behave similarly to quinol, but since their oxidation products are generally coloured, the blue benzidine mixture becomes colourless for an instant only and then the solution assumes a lavender, green, red, or brown hue, according to the phenol chosen. This colour slowly changes, and as the benzidine blue returns it becomes masked, and finally overpowered by the blue.

The phenols experimented with include *p*-cresol, orcinol, guaiacol, α- and β-naphthol, thymol, pyrogallol, resorcinol, phloroglucinol, saligenin, phenol, methyl quinol, dimethyl quinol, etc.

The list comprises certain phenols which usually do not give a colour reaction with oxydase, *e.g.*, methyl quinol. Even with α-naphthol the normal lavender oxidation coloration is much more intense when produced in the presence of other phenols.

The behaviour of methyl quinol deserves special mention in that it affords the basis for our hypothesis as to the production of serial colours in flowers. With oxydase, methyl quinol gives no colour reactions; but if a little benzidine be added to the colourless solution the latter takes on a deep and persistent carmine colour. The blue benzidine pigment acts catalytically as an intermediary for the transmission of oxygen to the methyl quinol; that is, it may in this respect, and in this case, play the part of an organic peroxide, and thereby achieve the oxidation of a substance (methyl quinol) which resists the action of oxydase and hydrogen peroxide.

The power of benzidine to transmit oxygen to methyl quinol and other phenols may be illustrated by making use of the oxydase present in the flowers, or other parts of plants. For instance, if the flower of a recessive white *P. sinensis* be treated with benzidine and hydrogen peroxide, the petals assume the blue-brown colour characteristic of the benzidine-oxydase reaction. If, however, benzidine and methyl quinol be added together with hydrogen peroxide a carmine coloration is produced.

A similar oxygen transmitting power on the part of benzidine is exhibited in the behaviour of the white flowers of *Lychnis coronaria.* Treated with benzidine alone the petals become brown; with α-naphthol they take on—albeit with extreme slowness—a lilac or lavender colour. If, however, the petals be treated with benzidine and α-naphthol they assume immediately a lilac colour which, taken in conjunction with the previous observations, indicates that benzidine facilitates the transference of oxygen from oxydase to α-naphthol.

In order to make clear the closeness of the analogy between the oxydase-benzidine and oxydase-benzidine-methyl quinol reactions on the one hand and those which lead to the production of the quinol colours—blue, red, and magenta—of such a plant as *P. sinensis,* it is necessary to give a brief account of the genetics of flower colour in this plant.

The flowers of *P. sinensis* stand in a definite and constant relation with one another. They form a series: recessive white, blue, red, magenta and dominant white. The biochemical nature of the whites has been described in an earlier communication (Part III).

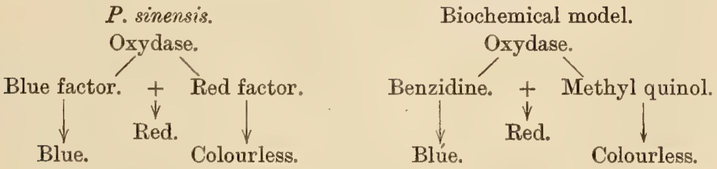
Of the coloured members of the series blue is recessive to both red and magenta, and red, which is dominant to blue, is recessive to magenta.

The Mendelian interpretation which fits the genetical facts is as follows:—

The character for blue flower depends on the presence of a single Mendelian factor. Red flowers also contain this factor and they contain in addition a factor for red which can produce its effect only in the presence of the "blue" factor. Similarly magenta flowered plants contain a magenta factor which when present together with the red and blue factors gives rise to the magenta character.

In the absence of the lower members of the series, colour is not produced and the colour of any flower is an indication that the series of factors is unbroken up to the factor for the colour character manifest in the flower.

We have thus a striking parallel between the colour series in *P. sinensis* and that which occurs with benzidine and methyl quinol. The closeness of the parallel is indicated thus:—



The peculiar behaviour of the red factor, first in failing to induce colour formation in the absence of the blue factor, and, second, in masking completely by a red pigment the activity of the blue factor, is to be accounted for thus:—The red factor determines the formation of a specific substance—perhaps of the nature of a phenol. That substance is not oxidised directly by the oxydase of the flower, but in the presence of the "blue" factor this specific substance receives oxygen from the blue pigment produced by the agency of that factor, and, in consequence, the blue pigment is reduced to the state of a colourless chromogen. The observations recorded on p. 126 lend additional support to this hypothesis. It is there observed that various phenols intensify, though they may not change, the colour produced by the action of oxydase on artificial chromogens. On the practical side it is also known that intensifiers of pigment exist, that they possess the power of converting a pale into a deep shade, and that they behave each as a unit character. On our model it seems reasonable to assume that an intensifier is a phenolic or similar substance, and that the factor for an intensifier means the power of the cell to produce that substance.

Lastly, on the basis of this hypothesis we have a plausible explanation of the fact that many oxydase reagents, though they give good "bundle" reactions, fail to reveal the presence of oxydase in the epidermis. The vascular tissues contain considerable stores of oxydase and oxygen-carrier, and hence, through the agency of the carrier, oxygen is transferred to α-naphthol or similar "artificial chromogen." The epidermal tissue contains only a small quantity of the carrier of oxygen, and hence, in spite of the presence of oxydase, α-naphthol and similar artificial chromogens remain unoxidised in this tissue.

Conclusions.

1. The pale yellow sap colour of the petals of the wallflower is a mixture of hydroxyflavone glucosides. The glucoside mixture is hydrolysed readily by heating with mineral acids and more slowly by emulsin of almonds. The hydrolysed product if reduced and subsequently oxidised yields a red pigment.

2. The fact that flowers containing similar soluble yellow pigments may be caused, by suitable chemical treatment, to yield a red pigment, suggests that red mutations should be of possible occurrence in such species.

3. The formation of pigments, as the result of oxidation by oxydase of the hydrolysed products of glucosides, is determined by the presence of amino-compounds and is of very general occurrence. The behaviour of the glucoside arbutin (see p. 121) makes it probable that many of the pigments and odorous substances formed during the ripening of fruits arise as results of reactions of this type.

The pigments of plants may be classified provisionally as follows:—

I. Plast Pigments—

a. Chlorophyll pigments contain	C, H, O, N
b. Carrotene contains	C, H
c. Xanthophyll (oxidised carrotene) contains	C, H, O

II. Sap Pigments—

- *a.* Yellow. Hydroxyflavone glucosides or derivatives thereof contain .... C, H, O
- *b.* Red, *e.g.*, of wallflower (see p. 117). Products of the action of oxydase on hydroxyflavone glucoside derivatives contain.. C, H, O

  [Whether all anthocyan pigments are of this type is unknown.]
- *c.* Red and brown, *e.g.*, of plum. Substances produced by the oxidation of phenols in the presence of amino-acids contain .... C, H, O, N
- *d.* As suggested in Section 3, the so-called anthocyan pigments (red and magenta) of flowers may arise as the result of the oxidation of phenol brought about by an organic oxygen carrier; contain ...... C, H, O

4. The benzidine-methylquinol-oxydase reaction (p. 128) provides an analogy with the II*d* type of pigment formation, and suggests the hypothesis that the higher members of a flower colour series (see p. 129) owe their origin to the presence with the lower members of specific substances which, acting as receivers of oxygen, reduce the pigments characteristic of the lower members of the colour series, accept oxygen, therefrom, and thereby become oxidised to pigments of specific colour.

On the Question of Fractional Activity ("All or None" Phenomenon) in Mammalian Reflex Phenomena.

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(Communicated by Prof. C. S. Sherrington, F.R.S. Received June 17,—
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(From the Physiological Laboratory, University of Liverpool.)

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I. *Introduction.*

At the present moment the question whether or not there is a state of "all or nothing" activity in reflex arcs seems to be raised, and it is one of importance to the future of investigation of the functions of the nervous system.

Of the two views which may be held regarding the manner of the activity of reflex arcs one is that in which it is supposed that the efferent neurone may react with different degrees of intensity in different reflex activities, and that the afferent neurones may play with different degrees of intensity upon efferent neurones or upon interposed neurones.

The other view, which seems now to be dawning, is one in which it is supposed that the efferent neurone has no grading in the intensity of its activity—it either reacts maximally or not at all; and if this be demonstrated it may perhaps be inferred that the afferent neurones act in a similar manner—that is, that their activity is either "all or none."

It is obvious that, if either of these views is shown to be the correct one, the course of research will in the future be modified.

II. *The Experimental Evidence.*

There is, at present, little direct experimental evidence bearing upon the question of "all or nothing" activity in reflex arcs.

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* The expenses of this research have been defrayed by a grant from the Carnegie Trust.

But in 1902 Gotch* showed that the electric organ of Malapterurus, which is served by a single efferent fibre, as compared with that of Torpedo, which is served by many fibres, has a much smaller range of reactions to reflex excitation. He yet states that within very restricted limits the organ shock is slightly augmented when an effective stimulus applied to its nerve is increased in intensity. He also notes that the initial shock reflexly evoked is variable in intensity, but he states that a considerable factor in this variation of intensity is probably fatigue of the nerve endings in the organ.

There is, however, some evidence which seems to show that the efferent nerve fibre and the effector organ (skeletal muscle) when stimulated artificially by faradic shocks may respond in an "all or nothing" manner to the peripheral stimulus.

In 1905 Keith Lucas† showed that when the exciting current which is applied to the skeletal muscle of the frog is gradually increased in strength the contraction of that muscle increases, not *pari passu*, but in abrupt steps.

Four years later the same investigator showed‡ that in the frog's cutaneus dorsi muscle, there is an "all or nothing" contraction of the muscle fibres in response to stimulation of the efferent nerve fibres—submaximal contraction of the whole muscle being due to a maximal contraction of less than the whole number of constituent muscle fibres.

Vészi, in 1911,§ made a curious observation which seems to throw doubt upon the "all or nothing" phenomenon in reflex conduction. He found, in the de-afferented frog, that in the state of strychnine poisoning there is an "all or nothing" contraction of gastrocnemius in response to stimulation of the cut afferent roots. A threshold stimulus evokes a maximum muscular response. But when this has fatigued by repetition of stimulation, a stronger stimulus again gives a maximum response.

In the following year the same observer|| came to the conclusion that the fresh amphibian efferent nerve-fibres follow the "all or nothing" rule; but that when fatigued the value of the excitatory process varies with the value of the exciting stimulus.

Slightly more recently Adrian¶ has investigated this question. He finds that a propagated disturbance in the efferent nerve fibres of amphibian nerve which has been reduced in magnitude by passing through a region of

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* 'Journ. Physiol.,' 1902, vol. 28, p. 395.

† 'Journ. Physiol.,' 1905, vol. 33, p. 125.

‡ 'Journ. Physiol.,' 1909, vol. 38, p. 113.

§ 'Zeitschr. für allgem. Physiol.,' 1911, vol. 12, p. 358.

|| 'Zeitschr. für allgem. Physiol.,' 1912, vol. 13, p. 321.

¶ 'Journ. Physiol.,' 1912, vol. 45, p. 389.

decrement regains its original size when it emerges into normal tissue. He points out that this favours the supposition that the relation between the disturbance and the strength of the evoking stimulus is of an "all or nothing" character.

Quite recently Mines* has given a description of experiments which suggest either that there is no gradation in the response of efferent amphibian nerve fibres to graded stimuli consisting of single induction shocks, or that the smallest excitation is capable of exciting any neuro-muscular synapse which can be excited by single impulses.

These various experiments certainly seem to point to the conclusion that the response both of the peripheral efferent nerve fibres and of the muscle fibres of the skeletal muscles is of an "all or nothing" character when the exciting stimulus is an artificial electrical one. And there is a temptation to argue from this that the activity of the same efferent nerve fibres and skeletal muscle fibres in the less artificial reflex excitation is also of an "all or nothing" character.

If this view be taken we must suppose the efferent neurone to discharge maximally or not at all. We must look at the reflex mechanism as one split longitudinally into units (as indeed we do look at it), which are each either maximally active or inactive, but never of intermediate activity. We must suppose that the grading of the muscular response is due to the differing proportions of its component units which at any one time are in action. But we must even then admit that a certain sort of grading of activity may occur even in one efferent neurone—for it might be supposed that the discharges proceeding from it might vary in frequency.

A subsidiary question is that of the possibility of a similar "all or nothing" character in the activity of the afferent neurones.

III. *Objections to "All or Nothingness."*

At first sight it might seem that a strong objection to the "all or none" character of afferent activities is before our eyes on any clear and moonless night. The stars appear to be of very different brightness, although the size of their images upon the retina is almost infinitely small and, theoretically at any rate, must be looked upon as stimulating only one retinal element each. It might seem that it is hardly possible to explain the number of distinguishable brightnesses as due to different numbers of retinal elements stimulated—for instance, are as many as ten stimulated in the case of a bright star, and as few as one in a star on the limit of visibility?

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* 'Journ. Physiol.,' 1913, vol. 46, p. 1.

Plausible as this objection to the "all or none" character of a certain specific afferent activity seems, it must be remembered that the imperfections of the eye may condition irradiation and halation sufficient to allow of the explanation of the phenomenon on the "all or nothing" principle.

But again, a wide range of touch sensations of different intensities may be obtained on stimulating the one point on the skin with stimuli of different values—and this even when the area of skin stimulated is supplied by a single afferent nerve. Here there seems to be no "all or none" response to graded stimulation.

Yet again, Sherrington* found for the scratch-reflex of the spinal dog that a dozen or more grades in the reflex response might be obtained on graded punctiform stimulation of the same skin-point—that is, of the same afferent nerve fibre.

These two latter observations seem to point very strongly to the conclusion that there must be a grading of the response, at any rate, in certain varieties of afferent nerve fibres of the mammal in reply to graded intensity of stimulation. And when we examine the possibility that the reflex discharge of the efferent neurone is of an "all or none" character certain difficulties are presented.

In the first place we have Sherrington's† statement that in a reflex response of the muscles of the hind limb of the mammal all the contractors are active in the minimal reaction, and that grading of the intensity of the reaction in response to grading of the intensity of the stimulus is accomplished by an increase in the activity of each contractor. If, however, the activity of each individual contractor is conditioned by a fractional activity of its fibres, it is, at any rate, strange that the thresholds of the most excitable fractions should be the same in all the contractors.

Secondly—perhaps not a very grave difficulty—there is the question of "tonus." This slight contraction on the "all or none" theory must be looked upon as due to the activity of a few of the muscle fibres only. But no sagging or wrinkling is seen in parts of a tonically contracted muscle, and the pull of the muscle where the tendon is a broad one is not seen more at one side than another.

Thirdly—and a more formidable difficulty—the "beats" of the scratch-reflex are usually "incomplete." That is to say, the flexor (for instance) exhibits a series of partial relaxations and reconstitutions of contraction. These may be looked upon as conditioned by a series of incomplete refractory phases. Now under an "all or nothing" theory each partial relaxation must be looked upon as produced by the "complete" relaxation of a proportion of the muscle fibres—or by the "complete" inhibition of a proportion of the efferent neurones. But Sherrington* has shown that the refractory phase extends over the whole centre—just as Zwaardemaker† showed the same phenomenon for deglutition. As regards this, the centre is therefore a unit, and in some manner innervated as a whole from each afferent neurone. It is, however, impossible to suppose that each afferent comes into direct contact with each efferent in the mechanism; and under any other supposition it is extremely difficult to realise the meaning of this unity if the efferent discharges have an "all or none" character.

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* 'Journ. Physiol.,' 1906, vol. 34, p. 1.

† 'Journ. Physiol.,' 1910, vol. 40, p. 28.

IV. *Methods here Employed.*

If a skeletal muscle exhibits a larger number of degrees of contraction than there are efferent nerve fibres running to it, there must be a very strong supposition that the reflex response has not an "all or none" character. In such a case the deduction from the result would not need to be influenced by the number of afferent fibres stimulated.

Unfortunately, the large number of efferent fibres which supply most of the skeletal muscles makes this experiment in their cases impossible.

In the cat, however, a most beautiful muscle in the hind limb—tenuissimus—seems almost to have been made for this experiment.

This muscle is a thin band of only 2 or 3 mm. in breadth, but many centimetres long. It arises from the caudal vertebræ at the root of the tail, and passes down deep in the thigh, until it ends in the leg by blending with the insertion of biceps. For the upper part of its length it lies near the great sciatic nerve, from which, near its middle, it receives its nerve supply. Occasionally it receives more than one nerve twig, and, in any case, its chief nerve divides into two branches (occasionally into more than two) before it reaches the muscle. The number of nerve fibres in each of these branches is small.

The cats used were decerebrate and low spinal. All the muscles of the left hind limb were destroyed by motor paralysis. In the right hind limb all were destroyed save tenuissimus. The great sciatic nerve was ligatured after it had divided into external and internal popliteals. The biceps muscle was divided transversely to its length about the middle, and tenuissimus was thus exposed.

All but the uppermost of the branches of its motor nerve were severed, and the muscle was cut across a little below the point where its nerve reached it. A fine silk thread connected it to the recording lever. This was a fine heart lever pivoted on agate cups.

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* 'Journ. Physiol.,' vol. 31, 'Physiol. Soc. Proc.,' March 19, 1904; 'Journ. Physiol.,' 1906, vol. 34, p. 1.

† 'Archives Internat. de Physiol.,' 1904, vol. 1, p. 1.

A fine silk thread was placed loosely round the intact branch of the motor nerve. Electrodes were placed on the great sciatic nerve, as far as possible peripheral to the point where the motor nerve left it for tenuissimus. All the exposed surface was then covered, but tenuissimus itself was left free and uncovered.

The reflex stimulus—faradic shocks, 30 per second—was applied for one second every minute. The mechanical responses were recorded upon the slow drum, but sometimes every 10th response was recorded on a faster drum. The intensity of stimulation was varied by changing the angle between primary and secondary coils degree by degree; sometimes also by sliding up the secondary millimetre by millimetre. In some cases the strength of stimulation was increased from a minimum, sometimes it was decreased from a maximum. Between stimuli the muscle was carefully covered up.

V. *Results.*

The observations here described rarely lasted for less than an hour—during which time a reflex contraction was recorded every minute. In these circumstances it was found that a certain deterioration of the preparation occurred—so that a direct muscular contraction was smaller at the end of the series than was one taken with the same strength of stimulation at the commencement of the series.

If a series of reflex contractions commenced with strong stimuli, and was continued with ever weaker stimuli, it is probable that a grading due to muscular "fatigue" might add itself to the true reflex grading. There might appear a larger number of "steps" than were actually conditioned by a grading in the efferent nerve.

In these experiments, therefore, the reflex stimuli were varied in the reverse order—that is, starting with subminimal stimuli, and gradually increasing the strength. Before and after each series a direct muscle contraction was registered, and, as in every case the contraction was smaller after the series, the number of different degrees of reflex muscular contraction registered was possibly less than the actual number. That this was so was also shown by the fact that, at the commencement of each experiment, there was a greater range of reflex contractions in a "quick" series (that is, one in which the graded stimuli were of widely differing intensity) than in the subsequent "slow" ascending series of reflex contractions, from which the estimation of the number of grades of contraction was made.

At the end of each experiment a maximal reflex contraction was registered. The remaining motor twig to tenuissimus was then divided, and the same reflex stimulus was again applied. If there was no muscular response it was assumed that that twig contained all the remaining motor fibres, and these were counted after staining with osmic acid. The number of different heights of reflex contraction was then counted and compared with the number of nerve fibres. Differences in height of under 0·5 mm. were neglected, and the heights were measured from the level of contraction which obtained at the commencement of each reflex contraction.

The following table gives details of six experiments:—

No.	Fibres.	Grades.	Kind of series.
1	31–33	27	Ascending series.
2	24–27	47	Descending series.
3	42	40	Ascending series.
		54	Descending series.
4	30	27	Ascending series.
5	48	28 (+21 ?)	Ascending series (broken).
6	28	31	Ascending series.

In Experiment 5 the series was taken in groups. Thus first a group of 10 closely graded stimuli was registered. The stimulus was then increased 10 times more than the increase between each pair of elements of this group, and a second closely graded group was registered. Again the stimulus was more greatly increased, and a third closely graded group was registered—and so on. As in each group there were about seven different grades amongst the 10 contractions, and as there were three "gaps" it is reasonable to suppose that about 21 grades should be added. The difference in height between the end contraction of a group and the commencing contraction of the next group was nearly the same (being sometimes greater) than that between the initial and terminal contractions of a group.

It will be observed that there are more grades in a descending series than in an ascending one. If the least favourable kind of series—that is, the ascending—be taken, it is found that the number of grades of reflex contraction corresponds fairly closely with the number of nerve fibres in the efferent nerve. It is sometimes a few more and sometimes a few less.

If this be the case, then there are more grades of reflex contraction than there are efferent nerve fibres.

For, in the first place, the presence of deterioration of the motor response probably means that there should have been more grades than were actually recorded.

And secondly, not all the fibres in the efferent nerve are efferent nerve fibres. A certain proportion of them are afferent nerve fibres from the sensory end-organs in the muscle. If tenuissimus conforms to the ordinary rule, about two-thirds to one-half only of the fibres in the motor nerve are efferent—for that is the proportion which Sherrington* found for the monkey and cat.

VI. *Conclusions.*

The experiments here described seem to show that the number of different mechanical responses with which a specific individual muscle (a flexor) answers certain reflex stimuli (ipsilateral flexion-producing) of different intensities may be greater than the number of efferent fibres in the motor nerve. The differences in mechanical response are here measured by estimating the extent of the greatest shortening of the muscle during a reflex tetanus which lasts 1 sec.

On the assumption that each difference in shortening of a greater extent than 0·5 mm. in the tracing as magnified by the lever is a measure of a different degree of reflex activity, it would seem that the activity evoked reflexly in the efferent nerve fibres here investigated has not an "all or none" character; and that the discharge of any efferent neurone may be graded in resonance with graded afferent stimuli.

But it must be admitted that the mechanical response is a coarse indicator —even under the conditions here used. And it must further be admitted that even if there is a larger number of mechanical responses than is the number of efferent nerve fibres this does not of necesssity exclude the possibility of an "all or none" character in reflex efferent discharges.

It is possible, for instance, to look upon the efferent part of a system as composed of three longitudinal parts—A, B, and C—each of which consists of efferent neurone and subservient muscle fibres. Three graded afferent stimuli might discharge: the first, A; the second, A+B; the third, A+B+C. In such an arrangement it would be expected that there would be three distinct and separable grades of mechanical response. But it is also possible that a series of graded afferent stimuli might discharge: the first, A; the second, A+B; the third, A+C (but not B); the fourth, B+C (but not A); the fifth, A+B+C. In such a case there would, therefore, be five possible mechanical responses if the muscular elements were of different efficiencies. This is certainly a possibility, but it must appeal to us at present as being too artificial a possibility.

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* 'Journ. Physiol.,' 1894, vol. 17, p. 211.

It seems best at present, in view of the difficulties met with in assuming an " all or none " activity, at any rate, in certain species of reflex arcs, to hold that the efferent neurones may discharge each with graded intensities.

If that be the case then it must appear that there is an essential difference between the activity of efferent nerves aroused by artificial peripheral stimuli and those evoked reflexly through the centres.

The question must arise whether this grading is one in which the amplitude of the discharge of each neurone may be varied, or whether the grading is produced by different speeds of repetition of discharges, the amplitudes of which are not varied.

In the latter case an explanation is offered only if the mechanical response varies with variation in the speed of repetition of nerve impulses.

That this is indeed the case Mines[*] has recently given some evidence to show. He points out that the ordinary explanations of the greater tension produced during tetanus than in single muscular twitches do not meet the case. He notes, for instance, that the fact that the tension set up in amphibian muscle in response to more rapid stimuli is greater than that set up in response to less rapid stimuli (which yet are sufficiently rapid just to give complete fusion) is not explained on the von Frey hypothesis.

One more point. The aspect of the problem which here particularly interests us is the question of an " all or none " response of the efferent neurone to graded reflex stimuli. Even in the case of peripheral stimulation there is little or no evidence of an " all or none " character of the response to graded stimuli of the efferent nerve fibre considered as a unit. As Adrian[†] himself points out, his experiments seem to show that certain longitudinal units of conduction are characterised in their activity by an " all or none " response to graded stimuli, but there is nothing to show that these units are the nerve fibres.

If they are units of a smaller size than the nerve fibres the efferent neurone may still respond in a graded manner to graded stimuli, although the activity of the elements of the discharge may be distinguished by this " all or none " character. If this be the case the discharge of the efferent neurone might be graded in " steps " from zero to its maximum.

That either the reflex discharge of the efferent neurone has not the character of an " all or none " response to graded stimuli, or that the longitudinal units, the activities of which possess the character of an " all or none " response to graded stimuli, are smaller than the nerve fibre seems to be shown by the experiments here described for one specific reflex type.

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[*] 'Journ. Physiol.,' 1913, vol. 46, p. 1.

[†] 'Journ. Physiol.,' 1912, vol. 45, p. 389.

VII. *Summary.*

The mechanical response of tenuissimus—a flexor in the hind limb of the cat—to graded reflex stimuli (tetani, lasting one second) seems under certain conditions to exhibit grades of difference greater in number than the number of efferent fibres in the motor nerve which supplies it.

On the assumption that the differences here observed denote differences in the activity of reflex discharges, this seems to show that the discharge of the efferent neurone in a specific type of reflex activity has not the character of an "all or none" response to graded stimuli.

This does not, of course, exclude the possibility that within the neurone there are units, the activities of which have this character.

Experiment 24.10.12 (No. 5 in table). Decerebrate cat; a record of the mechanical responses of right tenuissimus obtained in response to graded reflex stimulation of the right great sciatic nerve. Cat decerebrated 10.45 A.M.

The series was started at 11.27 A.M. The reactions are obtained in response to tetani lasting 1 sec. (rate of stimuli 30 per second), and they are taken every minute. The electrical stimuli are graded at first—with the primary and secondary induction coils 150 mm. apart—by rotating the secondary coil and thus diminishing the angle between its axis and that of the primary by 1° for each reaction. Later in the series the electrical stimuli are graded by pushing the secondary coil 1 mm. nearer the primary for every reaction (the axes of the coils then are parallel). The series is broken into groups of 10, and between the groups the electrical stimulus was graded tenfold the grading between the elements of the group by 10° or 10 mm. Between the five final reactions the grading is also of this order. Beneath each tenth reaction the value of the evoking stimulus (either in degrees divergence of the secondary axis at 150 mm. distance between coils, or in millimetres distance of coils with axes in line) is recorded.

In the first group there are at least eight different mechanical grades.

In the second group there are at least seven different mechanical grades.

In the third group there are at least seven different mechanical grades.

In the fourth group there are at least five different mechanical grades.

For the fifth group at least two more grades may be added. This gives a total of at least 29 mechanical grades. It can hardly be doubted that about the same proportion of grades would have been present in the first three intervals. On the assumption that in each of these there were seven distinct grades the total number of grades for the series would be about 50. "Quick" series registered before and after this record showed deterioration. This deterioration probably hinders the number of evident grades of contraction in such series.

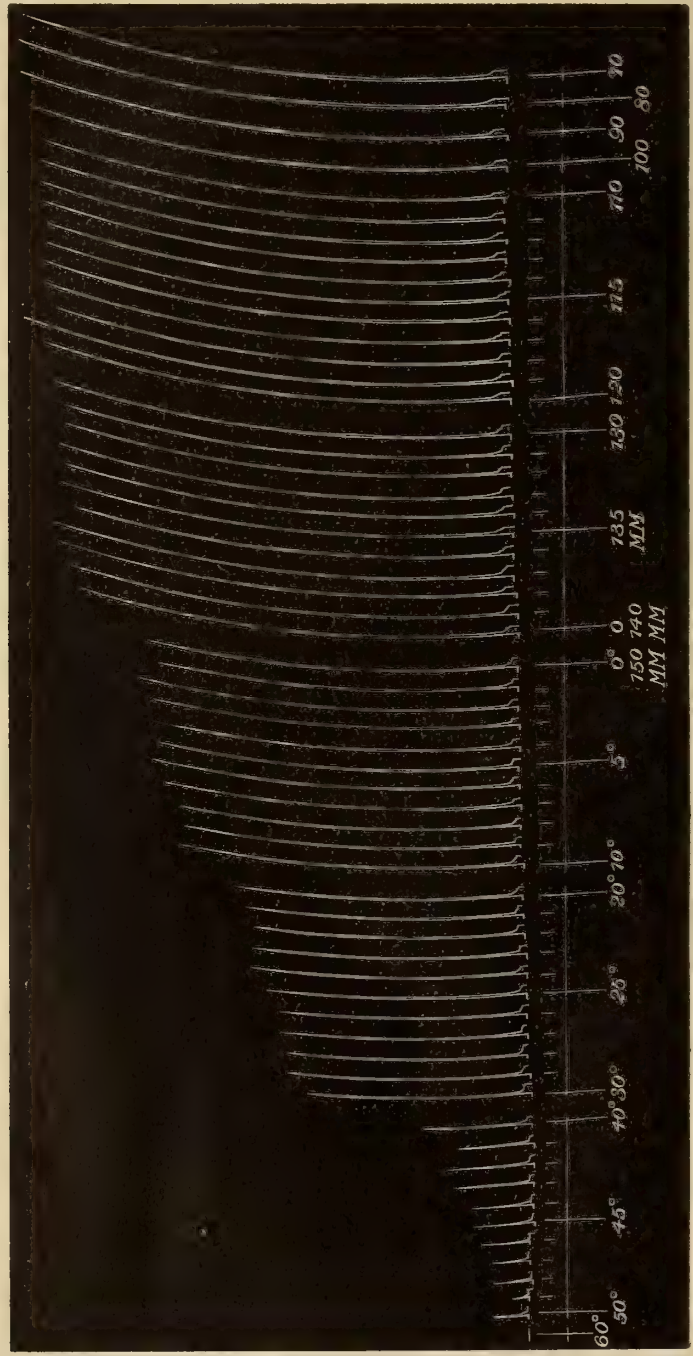

Fig. 1.

At the end of this experiment the remaining nerve twig of tenuissimus was divided. Thereafter all reflex contraction was abolished and there were 48 fibres in the twig. Of these probably not more than 32 were motor.

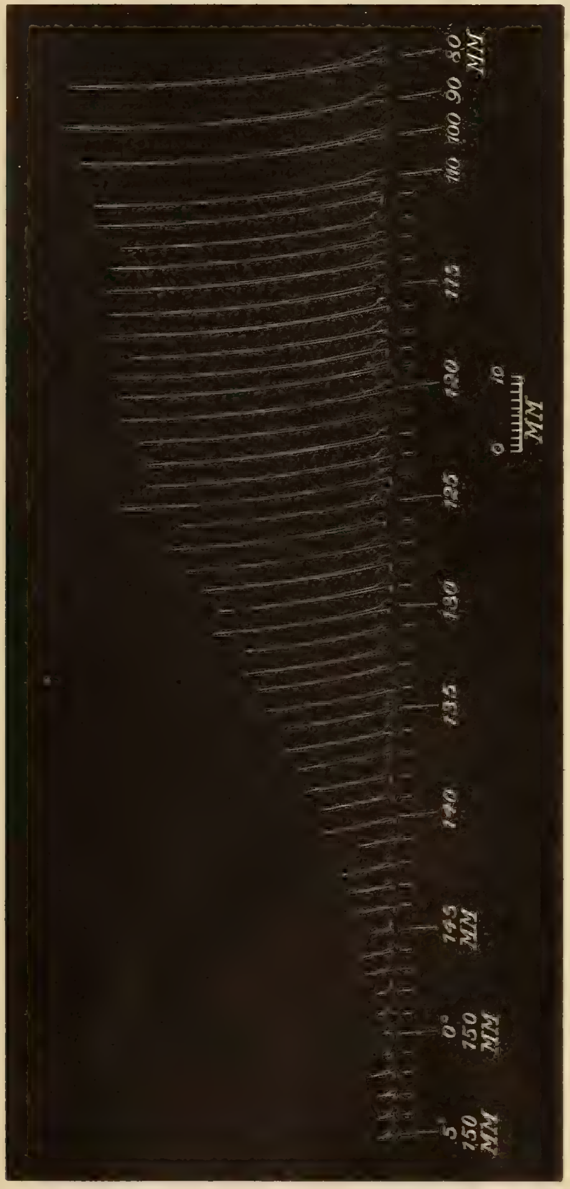

Fig. 2.

In this experiment, therefore, a number of motor fibres which probably did not exceed 32 conditioned reflexly a number of different mechanical grades of contraction which probably did not fall short of 50.

Experiment, 28.10.12 (No. 6 in table). Low spinal cat, cord divided at 11 A.M. A record similar to that reproduced in fig. 1, save that the series is complete. Series commenced at 12 o'clock, midday.

For the purposes of the experiment the following reactions may be considered to be of different grades: (marked by degrees) 1°, 0°; (marked by millimetres) 149, 148, 145, 147, 141, 144, 143, 142, 140, 139, 138, 137, 135, 136, 134, 133, 132, 129, 130, 128, 127, 126, 123, 121, 117, 120, 118, 116, 100, 90 = *circa* 32 grades.

Before this series was taken a "quick" series showed greater grading—between a minimum of 30° at 150 mm. and 90 mm. (coils in line). But in the figure the grading is over a range of about 150 to 90 mm. only.

After the series here reproduced was taken the remaining intact branch of the motor nerve was ligatured. Thereafter no reflex contraction was evoked on stimulation with the strongest stimuli here used. It was later found that there were about 28 fibres in that branch of the nerve. Of these probably about nine were afferent fibres.

Here, therefore, a number of grades which is probably about 30 was conditioned by the reflex activity of about 20 efferent nerve fibres.

On Postural and Non-Postural Activities of the Mid-Brain.

By T. Graham Brown (Carnegie Fellow).

(Communicated by Prof. C. S. Sherrington, F.R.S. Received July 21, 1913.)

(From the Physiological Laboratory of the University of Liverpool.)

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I. *Introduction.*

In the course of experiments in which the cerebral cortex of the monkey is stimulated, it is peculiarly noticeable that the activity of the cortex varies from time to time. That such variation should occur is by no means strange, in view of the difficulty of maintaining a constant depth of narcosis. But there are other variations which seemingly are not conditioned by variation of depth of narcosis. Thus it not rarely happens that, when the depth of narcosis is certainly a constant one, the motor cortex becomes suddenly inexcitable. This occurs, for instance, after a cortical discharge, which is followed by "epileptic" after-discharge. But it also occurs without any apparent preceding cause. Thus suddenly the cortical excitability becomes abolished—at any rate, to practicable strengths of stimulation.

This sudden loss of cortical excitability is a phenomenon of interest. It is accompanied by two marked states. Of these, the first is an anæmia of the cortex; the second is a maintained postural contraction of certain of the muscles of the limbs. The anæmia seems to occur over the whole of the small area of cortex—pre-central and post-central—usually exposed in these experiments. It causes a sudden change in appearance from the "raw ham" look of the cortex when it is in the most favourable condition for electrical stimulation to a pale "dead" look. The cortex blanches; it may be surmised that it faints.

The postural contraction of the muscles of the limb have most carefully been examined in the case of the contralateral arm. In that member the posture is one of flexion. The contraction of the flexors is a great one, and it may be an exaggeration of the slight postural contractions (both flexion and extension) which the arm always exhibits in changing degrees throughout these experiments. But, from these muscular activities, this state of greater contraction must be carefully distinguished. For, in the former, cortical stimulation is effective, and can abolish or augment the postural contraction; but, in the latter case, the stimulation of the cortex is ineffective.

When this curious phenomenon is examined, two points stand clearly out. There occurs a postural activity of the flexor muscles of the contralateral arm, and this is accompanied by anæmia and by inexcitability of the cortex. The similar state of inexcitability which is seen after post-stimulatory cortical epilepsy is also accompanied by blanching of the cortex, and often by maintained posture of the arm.

From these facts the conclusion may be drawn that the postural activity of the limbs in this state is conditioned by the activity of certain of the lower centres. The question arises—which are these?

II. *Methods Employed.*

The animals used in the present experiments were small monkeys: *Macacus rhesus*, *Macacus sinicus*, *Callothrix*, *Cercocebus æthiops*. They were kept unconscious throughout the whole experiment, and until they were destroyed at its conclusion. The procedures of decerebration, removal of the cerebellum, etc., were performed in the usual manners.

Stimulation of the various parts of the neuraxis was performed in the unipolar method. Two unipolar electrodes were used in order that two points might be simultaneously stimulated. These were on different circuits, and the two "indifferent electrodes" made necessary were applied one to either foot.

Stimulation of the peripheral nerves (ipsilateral and contralateral ulnars in the arm) was performed in the usual bipolar method.

For the proper examination of the movements of the arm in these experiments the movements of an extensor (humeral part of triceps brachii) and of a flexor (supinator longus) of the elbow were registered simultaneously. All the other muscles of the left arm and shoulder were destroyed by motor paralysis.

In the following descriptions the terms "ipsilateral" and "contralateral" are used in reference to the left arm—the former therefore meaning (here) "a point on the left side of the body," and the latter "a point on the right side."

III. *The State of the Monkey after Decerebration.*

After comparatively high decerebration (that is, when the neuraxis is divided across slightly anterior to the anterior colliculi), or even when the division is through the anterior colliculi, the animal is not perfectly immobile. When the depth of narcosis has shallowed the eyelids are open, and sometimes wide open. Winking frequently occurs, and the eyes sometimes are moved. From time to time the animal slowly changes its posture, the movements being like those of normal sleep. Owing to the fact that the animal in these experiments has been carefully covered and propped in a definite posture for the recording of the arm movements, it is not easy to describe those postures accurately. But, if the attention is confined to the movements of the left arm, it is seen that slow postural flexion and extension occur from time to time. The flexor thus may slowly contract, and, having reached its maximum of contraction, may there remain for many minutes if undisturbed. And, similarly, the extensor may at other times contract and remain contracted. The hind limbs may shew postural extension (Sherrington's "decerebrate rigidity") and, although the state of the hind limbs has not systematically been examined, it has seemed that they tend more frequently to demonstrate the extensor rigidity than do the fore limbs. From time to time the head is uneasily moved, and the animal seems to react (by closing the eyes) to loud and sharp sounds—although my evidence on this point is not very clear.

In short, the decerebrate monkey appears to be in a state which closely resembles that of light sleep, and the fact that, in this condition, these slow and maintained postural activities of flexion and extension in the arm may occur after decerebration shews conclusively that they are conditioned by centres below those in the cerebrum.

IV. *Electrical Stimulation of Regions in the Cross-section of the Mid-brain Dorsal to the Area of the Cortico-spinal Tract.*

Unipolar stimulation of the cross-section of the mid-brain at the level of the anterior colliculi—when applied at a point in an area which lies dorsal to that of the cortico-spinal tract—gives a definite movement of the arms. The focal point in this area—that is, the most excitable point in it—lies ventral to the central canal. The area includes that of the nucleus ruber and of the posterior longitudinal bundle.

Stimulation within this area upon one side of the mid-brain is accompanied by the assumption of a definite posture on the part of the animal. The back of the head is twisted towards the same side and the face away from it, the neck is bent concave to the same side (sometimes the face seems to be turned to the same side). The arm of the same side is flexed, that of the opposite side is extended. The lower limb of the same side is extended and the opposite one flexed (but at one period in an experiment in which this was usually the case I observed the ipsilateral hind limb to be flexed and the contralateral to be extended). The tail is bent to the same side. I have not been able carefully to examine the movements of the trunk.

When stimulation has ceased the posture is maintained. Thus if the attention be directed to the movements of the arm muscles alone it is found that the ipsilateral flexion (or contralateral extension) may outlive the evoking stimulus for several minutes.

1. *The Ipsilateral Reaction.*—When the movements of individual muscles in the arm are examined (*e.g.* fig. 2) it is found that stimulation of the ipsilateral area is immediately followed by a sharp flexor contraction. This soon attains a maximum at which it persists throughout the application of the stimulus. If extensor tonus is in being the flexor contraction is accompanied by reciprocal extensor relaxation. Sometimes during a long application of the exciting stimulus an extensor contraction—accompanied by slow flexor relaxation—may appear late in the period of stimulation. On withdrawal of the stimulus there is usually no relaxation of the state of flexor contraction, which then may persist for many minutes. This is the typical reaction, and by far the most common. But flexor relaxation occasionally occurs at the termination of stimulation, and this may be followed by an extensor terminal contraction which is comparatively well maintained. All these types of reaction have been seen 10 months after the division of all the posterior spinal roots supplying the left arm.

2. *The Contralateral Reaction.*—The result of stimulation of the contralateral area is to evoke a contraction in the extensor muscle (*e.g.* fig. 4).

This is accompanied by reciprocal flexor relaxation if there is flexor tonus at the commencement of stimulation. The extensor contraction is a more slow movement than the flexor contraction in the ipsilateral reaction. Having attained a maximum this persists throughout the period of stimulation and is continued after termination of stimulation as extensor postural after-discharge. This is often as well maintained as the flexor after-discharge in the ipsilateral reaction, but sometimes it dies away more rapidly. Occasionally augmented extensor contraction may be seen, and it sometimes happens that the terminal phenomena consist of extensor relaxation and flexor rebound contraction. This is rare and has occurred when there was considerable flexor tonus in being at the time of application of the ipsilateral stimulus—although even in these circumstances extensor after-discharge is the more common. The flexor rebound has been observed to change to extensor after-discharge after mesial longitudinal section of the mid-brain. Good after-discharge may be seen in the " de-afferented " condition.

3. *The Synchronous Compounding of Ipsilateral and Contralateral Reactions.*—The two reactions may obviously be synchronously compounded in such a manner that the ipsilateral interrupts a contralateral " background " or the contralateral an ipsilateral " background."

When compounded against an ipsilateral " background " (flexion) the effect of stimulation of the contralateral area (extension) is to produce relaxation of the " background " flexor contraction. This may be complete or it may be incomplete. When the relaxation is not complete it is found that stronger contralateral stimulation produces greater flexor relaxation during double stimulation. The flexor relaxation may be accompanied by reciprocal extensor contraction—which is, however, not so great in extent as that in the " pure " contralateral reaction (fig. 1). On the other hand there may appear no extensor contraction during double stimulation—even when that is present in the " pure " contralateral reaction. Although the extensor contraction is a slow one the flexor relaxation is a very rapid movement, but the latency of flexor relaxation is usually great. When the interrupting contralateral stimulus is withdrawn and the ipsilateral stimulus is continued there occurs a restitution of flexor contraction. This is usually a rapid movement even where there is a good extensor after-discharge in the contralateral reaction. The restituted flexor contraction may attain a level as great as that at the corresponding point in a " pure " ipsilateral reaction (fig 3, reaction " a "). Withdrawal of the ipsilateral " background " stimulus is followed by a flexor after-discharge just as in the " pure " reaction. In one instance an extensor terminal contraction and flexor terminal relaxation were seen. With the exception of the last phenomenon and of extensor

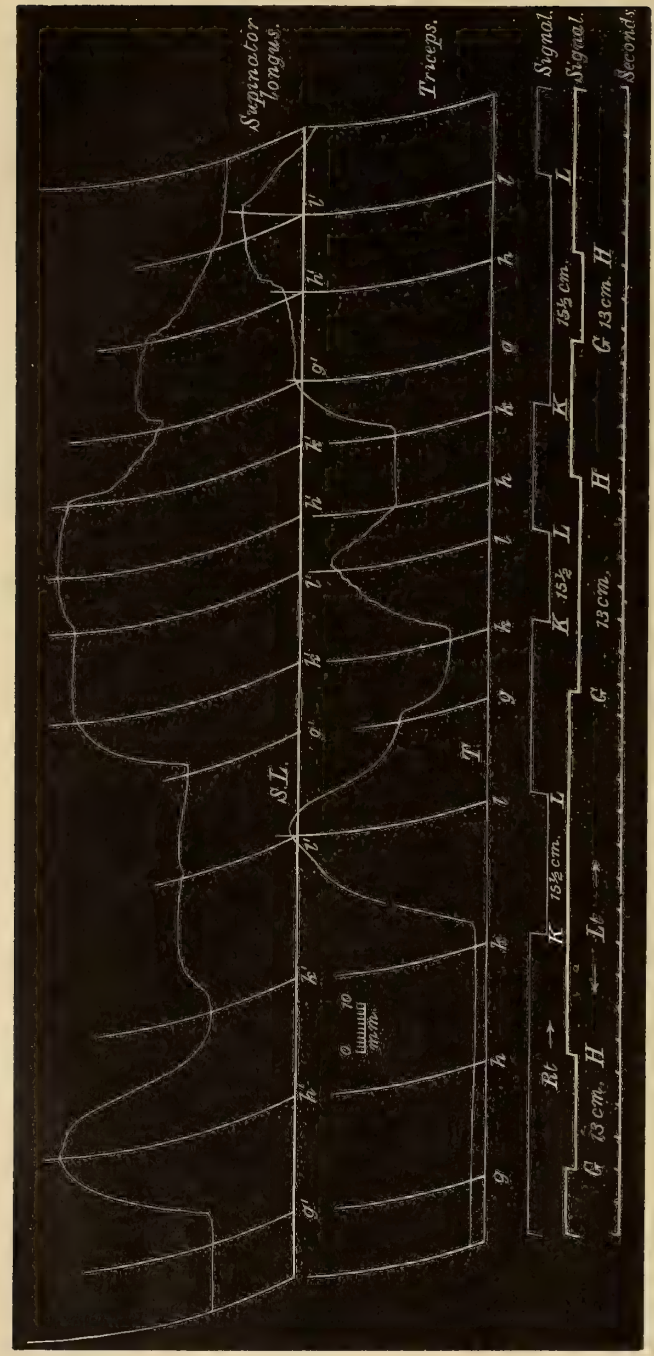


FIG. 1.

FIG. 1.—Experiment M, XXIX, record 327, 8860; 1.6.13.—*Macacus rhesus.* The record was obtained 1 hour after decerebration, and 12 minutes after mesial longitudinal

division of the mid-brain. The letters G–H (ordinates *g*, *g*′–*h*, *h*′) denote the period of stimulation of the ipsilateral "dorsal focal point" (posterior longitudinal bundle ?) in the cross-section of the mid-brain at the anterior colliculi. The letters K–L (*k*, *k*′–*l*, *l*′) in a similar manner denote contralateral stimulation. The upper record registers contraction (up) and relaxation (down) of the elbow flexor—supinator longus. The lower record registers similar movements of the elbow extensor—humeral head of triceps. Below these are the signal lines and a time tracing which registers seconds. A millimetre scale is reproduced, having been drawn upon the record before varnishing.

The first reaction is an ipsilateral one. On withdrawal of the stimulus the flexor after-discharge is extremely poor.

The second reaction is a contralateral one. Extensor contraction occurs. This is here accompanied by abnormal flexor contraction—not usually seen. There is an extensor after-discharge which is not well marked, but a sudden relaxation of this is seen at the commencement of the third reaction, which opens with the ipsilateral reaction.

In the third reaction the two stimuli are compounded synchronously with an ipsilateral "background." In the phase of double stimulation (*k*, *k*′–*l*, *l*′) there is extensor contraction and a partial and slight flexor relaxation. In this phase small rhythmic irregularities are evident in the two records. These are related to a slowing and deepening of respiration which then occurred. It will be observed that the extensor contraction is less in extent than that of the "pure" contralateral reaction (the second reaction of the record). On withdrawal of the contralateral stimulus there occurs flexor restitution and extensor relaxation. Flexor after-discharge occurs on withdrawal of the ipsilateral "background" stimulus, and is much better sustained than that in the preceding "pure" ipsilateral reaction.

In the fourth reaction of the record the "background" is contralateral (extension). The contralateral stimulus is applied in the flexor after-discharge of the preceding reaction and causes flexor relaxation (at *k*, *k*′). During double stimulation (*g*, *g*′–*h*, *h*′) there occurs a partial and slight extensor relaxation accompanied by reciprocal flexor contraction. Withdrawal of the ipsilateral stimulus is followed by flexor relaxation and extensor restitution of contraction. Withdrawal of the "background" contralateral stimulus is followed by extensor after-discharge.

This figure demonstrates the presence of flexor after-discharge in the ipsilateral reaction and extensor after-discharge in the contralateral; of the effects of compounding the two in temporal succession; and of the effects of synchronous compounding with ipsilateral and contralateral "backgrounds," all after mesial longitudinal division of the mid-brain.

contraction during double stimulation all these points have been observed in the "de-afferented" condition as well as in the "normal."

When compounded against a contralateral "background" (extension) an interrupting ipsilateral stimulus (flexion) evokes extensor relaxation and flexor contraction. Where the ipsilateral stimulus is comparatively weak the extensor relaxation may be incomplete (fig. 2). Where stronger it may be complete. Withdrawal of the interrupting ipsilateral stimulus is accompanied by a sharp relaxation of the flexor contraction. This may occur even when there is a flexor after-discharge in the ipsilateral reaction and when the contralateral "background" stimulus is ineffective. The flexor relaxation when the contralateral stimulus is effective may yet not be accompanied by restitution of extensor contraction. But that restitution may occur (figs. 1, 2). It then is a slow movement and closely resembles the extensor contraction in the "pure" contralateral reaction. Sometimes the flexor relaxation on withdrawal of the interrupting ipsilateral stimulus is a slow one (figs. 1, 2). Occasionally the flexor contraction may even be sustained after withdrawal of the ipsilateral stimulus (fig. 3). In such cases the flexor contraction is

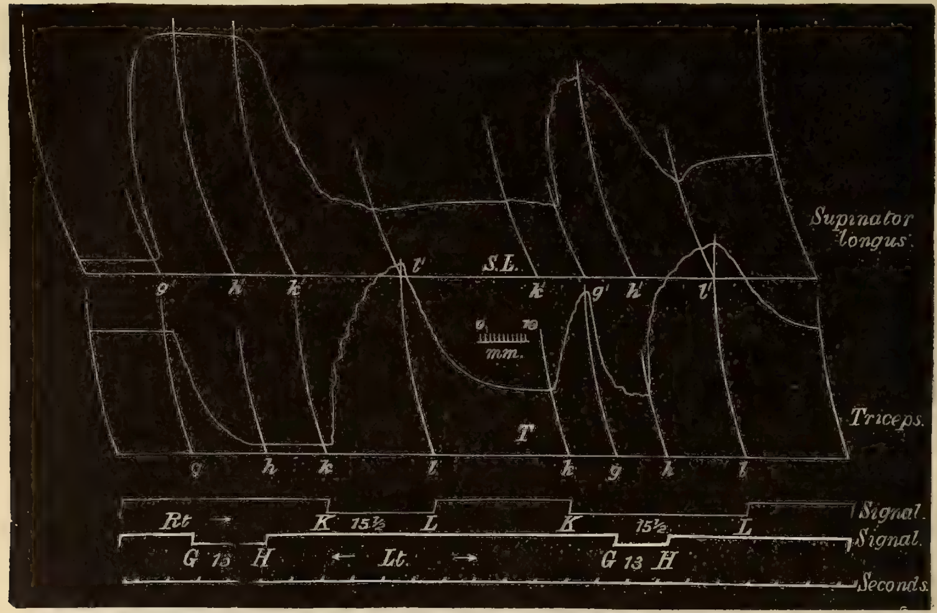


FIG. 2.—Experiment M, XXIX, record 327, 8855; 1.6.13.—*Macacus rhesus.* From the same experiment as fig. 1. This record was obtained 42 minutes after decerebration and *before* the mesial longitudinal division of the mid-brain.

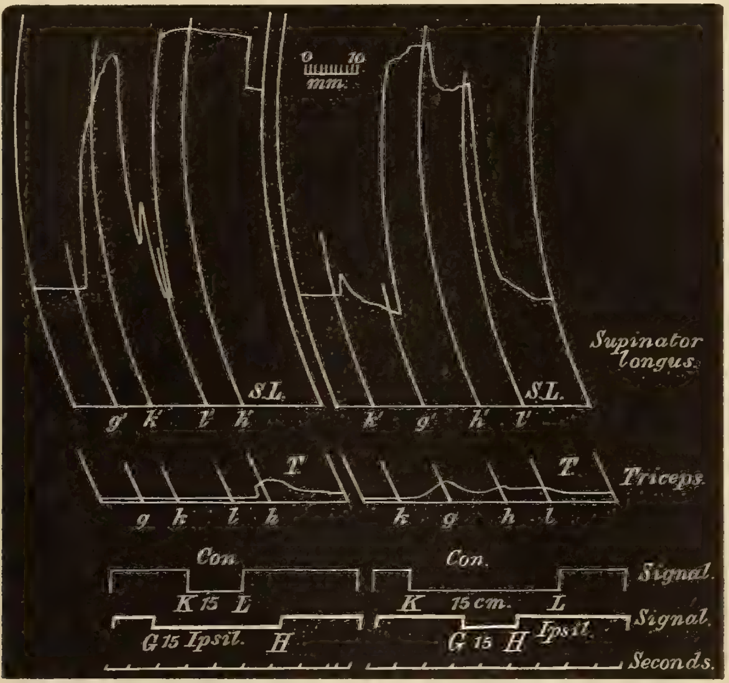
The first reaction is here an ipsilateral one, and it is applied during the presence of an extensor tonus from a preceding contralateral reaction. On stimulation extensor relaxation and flexor contraction occur. The ipsilateral reaction is followed by a good flexor after-discharge.

The second reaction is a contralateral one. Here there occur flexor relaxation and extensor contraction. The extensor contraction is again rhythmically notched. The extensor after-discharge is not well marked.

The third reaction is compound. The contralateral stimulus is first applied, and is then interrupted by an ipsilateral. During double stimulation (*g*, *g'*–*h*, *h'*) there is flexor contraction and extensor relaxation. The latter is not to so low a level, and the former is not to so high a level as those in the "pure" ipsilateral reaction. Withdrawal of the ipsilateral stimulus is followed by a slow flexor relaxation (it is usually more rapid) and by extensor restitution of contraction.

Compare this figure with fig. 1 (after mesial longitudinal division of the mid-brain). Here the effects of compounding the two reactions synchronously and in temporal succession are demonstrated as they occurred before the lesion.

not usually sustained on withdrawal of the contralateral "background" stimulus. Where extensor restitution of contraction occurs the withdrawal of the contralateral stimulus is followed by extensor after-discharge. In one case, where flexor relaxation occurred on withdrawal of the interrupting ipsilateral stimulus, there yet occurred flexor rebound on withdrawal of the contralateral "background" stimulus. There the same phenomenon occurred



a. *b.*

FIG. 3.—Experiment M, XXIV, record 311, 8362 ; 31.3.13.—*Macacus rhesus.* The dorsal spinal roots of the left (recording) fore limb divided in May, 1912. A reaction obtained 44 minutes after decerebration.

The first reaction (*a*) is a compound one with an ipsilateral "background." During double stimulation there is flexor relaxation, but no extensor contraction. The latency of the flexor relaxation is great. On withdrawal of the contralateral stimulus there is flexor restitution of contraction which occurs as a very sudden movement. On withdrawal of the ipsilateral "background" stimulus there is a marked flexor after-discharge. The sudden drop in the flexor after-discharge seen about 3 mm. before the final ordinate in reaction (*a*) occurred during a period of 15 seconds in which the kymograph was stopped—it therefore represents a very slow movement.

The second reaction (*b*) is a compound one in which the contralateral reaction is the "background." During double stimulation flexor contraction and extensor relaxation occur. But on withdrawal of the ipsilateral interrupting stimulus (at *h*, *h'*) there continues a flexor after-discharge. On withdrawal of the contralateral "background" stimulus this disappears.

in the "pure" contralateral reaction. With regard to the phenomena described in this paragraph there is little difference between the "de-afferented" and the "normal" conditions.

It therefore appears that the phenomena obtained when the two areas in the cross-section of the mid-brain are simultaneously stimulated closely resemble those obtained when the movements of two antagonists in response to peripheral nerve stimulation are examined. "Algebraic summation" seems to occur, and the phenomena which occur when the interrupting stimulus is withdrawn and the "background" still continued nearly approximate to those seen under similar conditions in the peripheral reflexes where the "pure" reactions are followed by good "after-discharge."

4. *The Compounding of Ipsilateral and Contralateral Reactions in Temporal Succession.*—Where the ipsilateral reaction is followed by flexor after-discharge and the contralateral by extensor after-discharge the two reactions may be compounded in such a manner that the one falls during the after-discharge of the other. If this is done the contralateral reaction (extension) at once produces a very sharp relaxation of the flexor after-discharge of the ipsilateral reaction. On withdrawal of the contralateral stimulus an extensor after-discharge is left in being, and this is at once reduced if the ipsilateral stimulus is repeated, and so on (fig. 2). If the contralateral stimulus is weak or of very short duration there may be incomplete relaxation of a flexor after-discharge. If the ipsilateral stimulus is weak there may be a partial relaxation of an extensor after-discharge, and this may thereafter be reconstituted.

5. *The Geographical Position of the "Focal Point."*—When stimulation applied to one or other side of the cross-section of the mid-brain gives one or other of these reactions it is usually found that the area from which they may be obtained is comparatively large. The minimal reaction may, however, be localised to a comparatively small area. This area ("focal point") lies about 3 to 4 mm. ventral to the dorsal surface of the mid-brain, and about 2 to 3 mm. from the mesial plane. The surrounding parts are inexcitable, except, perhaps, those immediately between the focal point and the mesial plane.

In one experiment in which decerebration was comparatively high—the division of the neuraxis passing just oral to the anterior colliculi—the focal point was found to be much more ventral than this, about 7–8 mm. from the dorsal surface. The ipsilateral reaction was of the usual type. The contralateral reaction gave relaxation of a flexor after-discharge (if that was in being) and extensor contraction, but on withdrawing the stimulus there was at once sharp extensor relaxation and a marked flexor rebound contraction. The mid-brain was then split in the mesial plane (the section was found to have passed out of the mesial plane into the left half of the neuraxis at the level of the posterior colliculi). Immediately thereafter the focal point was found to be in the usual more dorsal position. Ipsilateral stimulation gave the usual reaction, and contralateral stimulation gave the same reaction as before, save that there was marked extensor after discharge and no flexor rebound contraction.

V. *The Effect of Various Lesions.*

1. *Mesial Section between the Right and Left Halves of the Mid-brain.*—When the reactions are obtained from the dorsal focal point there may be no change in them after this lesion. The phenomena during the immediate and successive compounding of the two reactions may be the same as before (figs. 1, 4). The excitability may be depressed slightly, or it may remain unchanged, or it may even appear to be raised. The effects of mesial section in a case where the lower focal point was effective have been described in the previous section.

2. *Right Semi-section of the Mid-brain between Anterior and Posterior Colliculi.*—After this lesion it is found that the ipsilateral reaction (from the left side of the mid-brain) is unimpaired, but the contralateral reaction (from the right side of the mid-brain above the level of the semi-section) is abolished. The contralateral reaction may at once be obtained by stimulation of the caudal surface of the cut in the mid-brain.

3. *Division of the Right Superior Cerebellar Peduncle.*—This was found to have no appreciable effect upon the reactions and their compound effects. The experiments upon the cerebellar peduncles, in view of the effects of removal of the whole cerebellum, were not continued.

4. *Complete Removal of the Cerebellum.*—In several experiments the cerebellum has been completely removed. After this lesion there is at first no change in the two reactions. Flexor after-discharge follows the ipsilateral reaction and extensor after-discharge the contralateral, and the effects of compounding the two in temporal succession is the usual one (fig. 5). This may last for 30 minutes or more. Thereafter the flexor after-discharge disappears, the withdrawal of the ipsilateral stimulus being followed by sharp flexor relaxation. In one experiment the extensor after-discharge still persisted. No change in the excitability of the reactions may occur. In other cases the flexor after-discharge may disappear from the moment of removal of the cerebellum (the reactions have usually been tested within one minute of that removal). In one case the contralateral reaction reversed to flexion with the same strength of stimulus which before the removal gave extension. The procedure of removal of the cerebellum has been observed

to be followed by marked extensor tonus. In one case marked flexor tonus was present immediately after the removal.

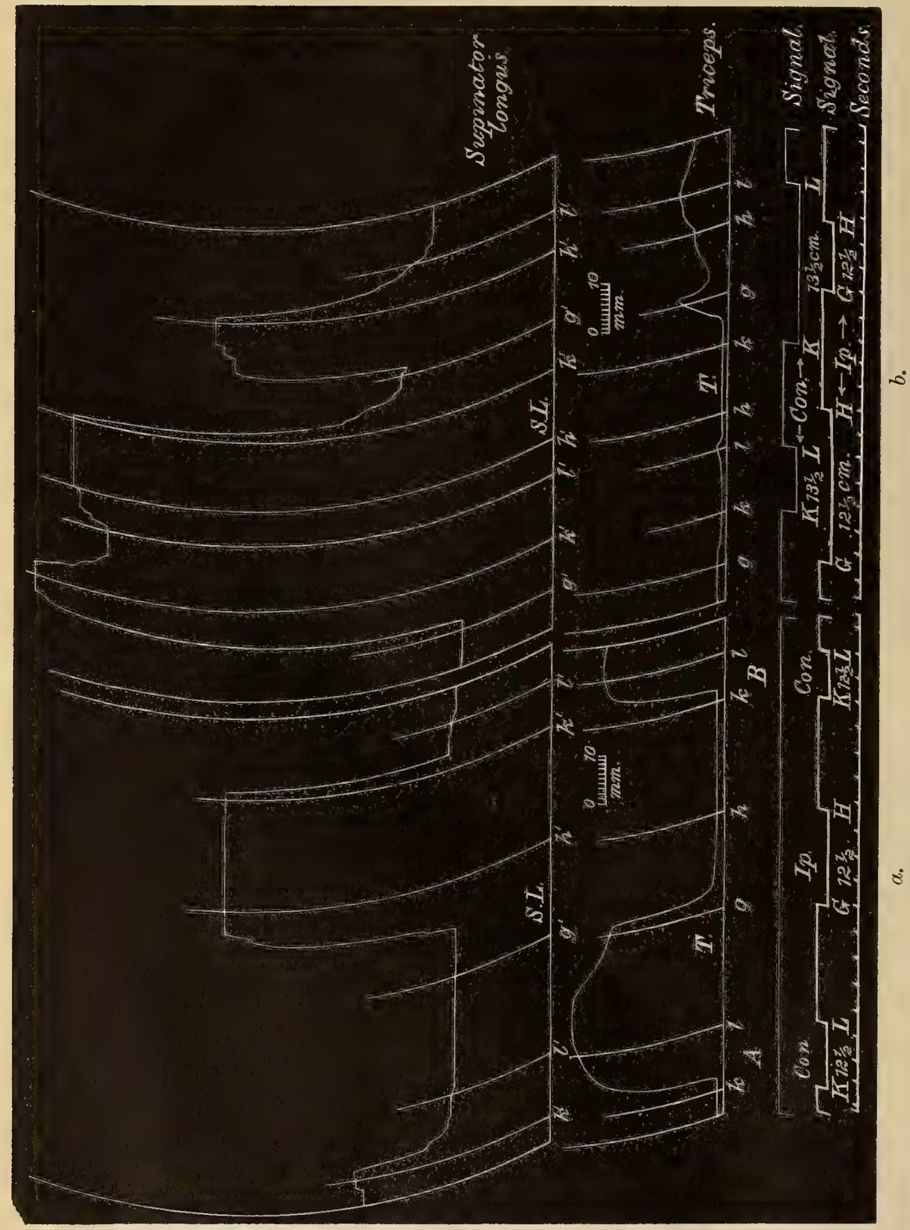


Fig. 4.—Experiment M, XXX, record 329, 8885; 6.6.13.—*Macacus rhesus.* The two reactions are obtained 50 minutes after decerebration, and 12 minutes after mesial longitudinal division of the mid-brain.

In *a* the first reaction is contralateral. Applied during a flexor after-discharge, it gives flexor relaxation and extensor contraction, and is itself followed by a marked extensor after-discharge. The second reaction is applied during this. It is an ipsilateral one, and gives extensor relaxation and flexor contraction, being followed by a well sustained flexor after-discharge. The third reaction is again contralateral, and repeats the phenomena in the first.

In *b* the two stimuli are synchronously compounded. In the first reaction the ipsilateral "background" shows but small relaxation during double stimulation. In the second the contralateral "background" is weak, and yet the flexor contraction of double stimulation is markedly smaller than that in the "pure" ipsilateral reaction.

This figure, and also fig. 1, demonstrates the antagonistic effects of the two stimuli after mesial longitudinal division of the mid-brain.

5. *Removal of Mid-brain.*—In one experiment in which flexor after-discharge in the ipsilateral reaction outlasted for some time the removal of the cerebellum it was found that it also outlasted removal of part of the

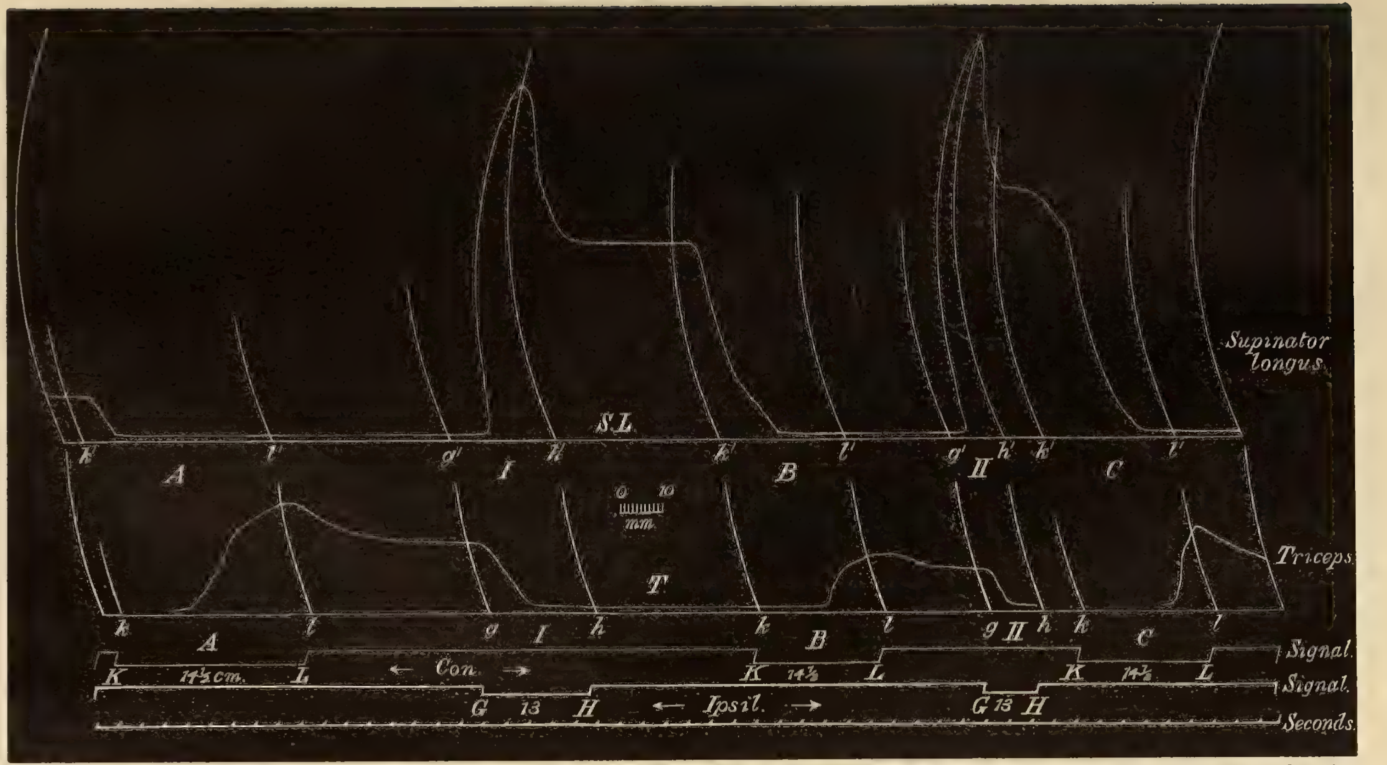


Fig. 5.—Experiment M, XXXV, record 337, 9094; 17.6.13.—*Callothrix.* The reactions are obtained 55 minutes after decerebration and 10 minutes after complete removal of the cerebellum.

Flexor tonus is in being at the commencement of the series. Reactions "A," "B," and "C" are contralateral. They demonstrate flexor relaxation and extensor contraction during double stimulation and extensor after-discharge after stimulation.

Reactions "I" and "II" are ipsilateral. During stimulation there is first relaxation of the extensor after-discharge contraction, and then flexor contraction. On cessation of stimulation there is a partial flexor relaxation which becomes arrested, and flexor contraction then persists as after-discharge.

This figure demonstrates—*after removal of the cerebellum*—(*a*) the persistence of flexor after-discharge in the ipsilateral reaction from the cross-section of the mid-brain, and the persistence of extensor after-discharge in the contralateral reaction; and (*b*) the persistence of the antagonistic effects of these two reactions when they are compounded in temporal succession.

mid-brain. The extensor contraction in the contralateral reaction had disappeared, but the ipsilateral reaction was untouched. The anterior colliculi were then first removed. Flexor after-discharge still occurred after the ipsilateral reaction. If the contralateral stimulus was applied during this flexor after-discharge there occurred flexor relaxation which continued after the cessation of the contralateral stimulus. When the posterior colliculi were then removed the flexor after-discharge in the ipsilateral reaction was not clearly marked, and the contralateral effect was not obtained (the contralateral reaction in response to weak stimuli appeared to be one of flexion). When the oral half of the pons Varolii was removed after a transverse section the reaction which accompanied stimulation of the ipsilateral area was not followed by any after-discharge. In another experiment the flexor after-discharge had disappeared from the ipsilateral reaction after removal of the cerebellum, and it remained absent after further removal of the mid-brain. Here also the contralateral reaction appeared to be one of flexion.

VI. *Electrical Stimulation of the Crus Cerebri.*

It may be suggested that the results above described might, in part at any rate, be due to stimulation of the crura cerebri by spread of current. That this is not the case is shewn by two observations.

Of these, the first is the fact that with the strength of stimuli used the ipsilateral crus cerebri is inexcitable as regards the movements of the arm of its own side of the body. The contralateral crus is excitable, but the movements of the elbow obtained from it are almost invariably flexion, whereas the contralateral reactions from the area in the cross-section of the mid-brain dorsal to it are those of extension. Further, there is an inexcitable field between the two.

The second observation is that the movements of flexion excited by stimulation of the contralateral crus are unlike those obtained on stimulation of the area in the ipsilateral side of the cross-section of the mid-brain. The crus flexion is almost always a slow one and "climbs" during the course of application of the stimulus, so that its maximum is almost always at the point of cessation of stimulation. Cessation of stimulation is followed by a very sudden relaxation, so that the arm "flops." This is extremely characteristic.

VII. *Compound Stimulation of Crus and the more Dorsal Excitable Area in the Cross-section of the Mid-brain.*

In these experiments the effects of compound stimulation of various kinds have been examined. Here there is not space to mention all of these, but the phenomena when crus is pitted against the excitable area which lies more dorsally in the cross-section of the mid-brain are of great interest.

1. *Immediate Compounding of Crus against Contralateral Reaction (Extension).*—The crus reaction used was the typical contralateral flexion. In one experiment ("de-afferented"), where the "background" of the compound reaction was that of the contralateral reaction (extension with extensor after-discharge) the interrupting crus reaction caused extensor relaxation accompanied by flexor contraction of the typical crus type. Withdrawal of the crus stimulus gave flexor relaxation and reconstitution of extensor contraction. Withdrawal of the "background" contralateral area stimulus was followed by the usual extensor after-discharge. When the contralateral reaction is made to interrupt the crus reaction a relaxation of the flexor contraction has been observed during double stimulation.

2. *Immediate Compounding of Crus against Ipsilateral Reaction (Flexion).*—Here when the ipsilateral reaction serves as the "background" there occurs flexor augmentation of contraction when the interrupting crus stimulus is applied. On withdrawal of that stimulus again, the ipsilateral reaction still continuing, the flexor contraction remains augmented. The subsequent after-discharge of the flexor on withdrawal of the "background" ipsilateral reaction may remain at this level.

3. *Compounding in Temporal Succession.*—When the ipsilateral reaction from the more dorsal excitable area in the cross-section of the mid-brain gives a marked flexor after-discharge and the contralateral crus stimulus is then applied, augmentation of flexor contraction may occur. But, on withdrawal of the crus stimulus, there is immediate and very sudden flexor relaxation—exactly similar to that which follows a "pure" contralateral crus stimulus. This phenomenon is the more remarkable in that it may occur where the flexor after-discharge after the ipsilateral reaction is of great extent and very great duration. It seems to indicate that the flexor relaxation after crus stimulation is, as it were, a positive phenomenon (fig. 6). This phenomenon has been observed in the "de-afferented" condition. It has also been observed after removal of the cerebellum.

VIII. *Stimulation of other Points in the Mid-brain and Hind-brain.*

In passing, I should like to note some effects of the stimulation of other points in the hind-brain and mid-brain.

In one experiment, where the level of cross-section in decerebration was just above the anterior colliculi, a curious bilateral kick-like movement of the lower limbs was obtained on stimulation of a small area on either side of the cross-section. This was to the side and dorsal in position, and it is possible that the reaction was a reflex in connection with the optical mechanism.

In several experiments it was found that unipolar stimulation applied to a certain area on the ventral surface of the exposed fourth ventricle evoked

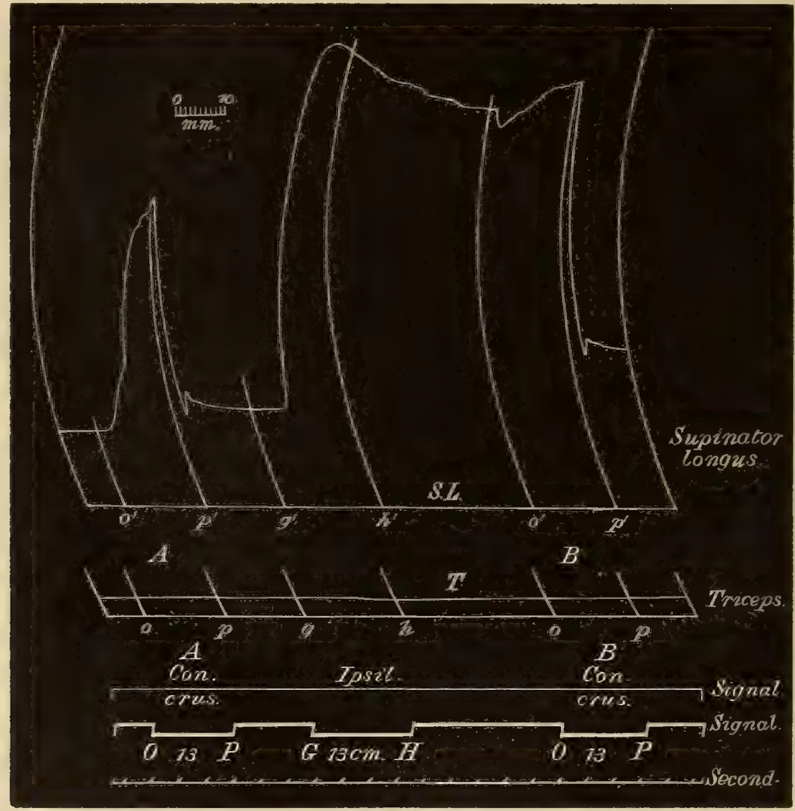


Fig. 6.—Experiment M, XXV, record 315, 8481; 1.4.13.—*Macacus rhesus.* All the dorsal spinal roots of the left (recording) fore limb divided in May, 1912. A record of the reactions from the ipsilateral area on the cross-section of the mid-brain and of the reactions from the contralateral crus cerebri obtained 1 hour and 8 minutes after decerebration.

The first reaction (O–P, ordinates *o*, *o'*–*p*, *p'*) is that from the contralateral crus cerebri (A). It demonstrates the typical "climbing" form of the contraction, and the sudden relaxation on withdrawal of the exciting stimulus. [It was not possible to induce an after-discharge by repeated stimuli in series.]

In the second reaction an ipsilateral "dorsal focal point" stimulus is applied, and gives the usual flexion followed by flexor after-discharge in this experiment that would last for several minutes if undisturbed.

In the third reaction (B) the crus stimulus is repeated during this after-discharge. It gives first a slight relaxation, and then an augmentation of flexor contraction. But, nevertheless, on withdrawal of the crus stimulus the sudden relaxation occurs as in the "pure" crus reaction. The flexor after-discharge is abolished.

flexion of the ipsilateral arm. This area lay about 3 mm. above the tip of the calamus and was near the mid-line. To stimuli of a strength which could evoke this, the surrounding regions were inert.

Stimulation of the nucleus cuneatus in the medulla oblongata gives flexion of the ipsilateral arm. Stimulation of the nucleus gracilis gives movements of the lower limbs. It has been found in some experiments that the flexion of the arm evoked by stimulation of the ipsilateral nucleus cuneatus is followed by marked flexor after-discharge. This may occur in response to very weak stimuli, and in these experiments the cerebellum had been removed. The after-discharge has been found to disappear after removal of all in front of the medulla oblongata, and after removal of the mid-brain alone.

IX. *Conclusions.*

The "dorsal focal point" in the cross-section of the mid-brain—the results of stimulation of which are here described—corresponds geographically with the cross-section of the posterior longitudinal bundle at that level. The "ventral focal point" (described for one experiment) corresponds geographically with the area of the nucleus ruber. The effective strength of stimulation of these areas is much greater than that necessary to evoke reactions from the motor nuclei or from the nucleus cuneatus.

In these circumstances there is a great risk of error due to spread of current. But the fact that the reaction from the dorsal focal point is abolished after lateral semi-section of the mid-brain behind the point stimulated, and that the reaction may still be obtained from the caudal surface of the semi-section, makes it extremely probable that the phenomena here described are conditioned by stimulation of the posterior longitudinal bundle. That they are apparently unchanged after mesial longitudinal division of the mid-brain helps to confirm this view. Is it possible that the stimulation of the "ventral focal point" is stimulation of the rubro-spinal tract? My evidence at present is quite insufficient to make this certain, but there are certain points of interest which may be noted. In the first place, the excitability of this point drops after mesial longitudinal division of the mid-brain, and this looks as if there was a decussation of the tract stimulated in the mid-brain itself. Another point which may be noted is that, whereas before this mesial longitudinal division the ipsilateral reaction of the ventral focal point was one of flexion, after that division it appeared to yield ipsilateral extension. As I have said, the evidence on this point is quite incomplete, but may the guess be hazarded that the crossed and uncrossed portions of the rubro-spinal tract subserve different functions?

To turn again to the dorsal focal point (posterior longitudinal bundle?), some phenomena of interest are presented. Of these the first is the characteristic tendency of the reactions to be followed by after-discharge (contralateral, extension; ipsilateral, flexion). The reflexes evoked are essentially postural. This attribute disappears neither after mesial longitudinal division of the mid-brain nor immediately after complete removal of the cerebellum. Postural tonus of a perfect maintenance may be evoked in the absence of the cerebellum by appropriate stimulation of these tracts. It is possible that their activity is one of the chief factors in the great postural reflexes, and that the cerebellum plays upon them but is not itself the originator of the postural after-discharges. That the after-discharges do slowly disappear after removal of the cerebellum may be due either to shock or perhaps to the removal of a function of the cerebellum in maintaining the proper activity of these paths and centres.

The second point of interest is the mutual antagonism of the right and left dorsal focal points. This, seen either in immediate or successive compounding of the two, does not disappear either after mesial longitudinal division of the mid-brain or after complete removal of the cerebellum. The point of common antagonism is below the mid-brain, and it may be surmised that it lies at as low a level as that of the spinal centres.

A third point of interest is that the reactions—with their typical after-discharges—may occur many months after division of the dorsal spinal roots of the arm. That is to say, appropriate stimulation in the region of the mid-brain may evoke an extensor postural tonus or a flexor postural tonus. Sherrington has found that the "decerebrate rigidity" which occurs after removal of the cerebrum does not occur in a "de-afferented" limb, but the fact that a condition which at any rate very closely resembles this state may be evoked in such limbs seems to point to the conclusion that the absence of this postural tonus in the decerebrate "de-afferented" animal is due to the failure of the ascending impulses from the limb which normally play—however indirectly—upon these mechanisms of the mid-brain, and that the mechanisms themselves if properly activated are still able to induce the tonus.

One point more may be referred to—the activity of the cortico-spinal tract. The activity of the posterior longitudinal bundle (?) seems essentially to be postural. That of the cortico-spinal tract seems essentially to be non-postural. When the stimulus is stopped the reaction at once fails, and that with great suddenness. When the flexion reaction of the contralateral crus cerebri is pitted against the flexor after-discharge of the ipsilateral dorsal focal point (posterior longitudinal bundle ?) the phenomena are of great interest. There occurs during stimulation of the crus immediate augmentation of flexion, and it might have been supposed that the flexor after-discharge remained unimpaired after withdrawal of the crus stimulus. But this is not the case. Immediate relaxation of flexor contraction occurs just as in a "pure" crus reaction. To this extent the crus flexion reaction seems to be antagonistic to the flexion reaction of the after-discharge in the dorsal focal point reaction. The non-postural cerebral activity seems to abolish the postural mid-brain activity and thus to leave, perhaps, a virgin field for any subsequent reaction.

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*Synthesis by Sunlight in Relationship to the Origin of Life. Synthesis of Formaldehyde from Carbon Dioxide and Water by Inorganic Colloids acting as Transformers of Light Energy.**

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At a discussion on the origin of life held by the joint sections of Zoology and Physiology of the British Association, at the Dundee Meeting, September, 1912, it was suggested by Moore that the first step towards the origin of life must have been the synthesis of organic matter from inorganic by the agency of inorganic colloids acting as transformers or catalysts for radiant solar energy. This suggestion was amplified and explained in a book written in November, 1912, by the same author.†

It is important to emphasise the point that in considering the origin of life in a world containing inorganic matter only, the nutrition of the first living structure on such a world must be carefully borne in mind. This observation is still true whether life is to be regarded as arising *de novo* on the planet, or as being borne there from some other planet as a germ from pre-existent life. No living organism such as a bacterium or mould which did not possess the power of transforming energy and of synthesising organic from inorganic matter could exist or flourish in total absence of pre-formed organic matter and must inevitably perish.

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* The cost of materials and apparatus for this research have in part been defrayed by a grant from the Government Grant Committee of the Royal Society, and in part by a donation from Mr. William Johnston, of Liverpool.

† Moore, 'The Origin and Nature of Life,' pp. 181–193, Home University Library. Williams and Norgate, London.

A substance acting as a transformer of light energy with accompanying synthesis of organic from inorganic matter now exists in our world, in chlorophyll, the green colouring matter of plants, and also allied bodies such as the blue-green colouring matter of the Cyanophyceæ possess a similar power. But both these substances are exceedingly highly organised and complex, quite unsuitable by their nature to be thought of as the first stage in the evolution of organic from inorganic matter at the dawning of life in a world hitherto devoid of anything organic.

The protoplasm of the living cell also is built up of the most complex organic compounds known to us such as could scarcely arise in an entirely inorganic world as the first step from inorganic to organic matter.

The first primeval step would appear to be indicated by the union of single crystalloidal inorganic molecules to form inorganic colloids, and that these meta-stable colloids acting on inorganic carbon compounds, such as carbon dioxide, in presence of water and sunlight, and taking energy from the sunlight, built up at first simple organic bodies, and now these in turn reacting with one another formed more and more complex organic compounds. In any such transformation external energy is necessary, because the reacting bodies, carbon dioxide and water, are fully oxidised, and must be reduced with evolution of oxygen and uptake of energy in what is called an endothermic reaction. To this reaction, the inorganic colloid plays the part of an activator or catalyst, the solar energy being converted into chemical energy of the organic compound, so serving as a reservoir of the energy necessary for the coming living organic world.

It was first suggested by Baeyer* that the initial stage in the synthesis of organic matter from inorganic by the green plant consisted in a reaction of carbon dioxide and water to produce formaldehyde and oxygen, the energy for the endothermic reaction being supplied from the energy of the light vibrations. This has been confirmed by delicate reactions, for although the change is a transitory one, the formaldehyde being condensed into other organic substances as it is formed, yet colour reactions for aldehydes are known so delicate that they will clearly indicate 1 part in 1,000,000 of aldehyde.

Any accumulation of formaldehyde would rapidly kill the living cell, and it is soon transformed into other products, but the colour tests are so delicate that its presence in traces has now been confirmed by several observers.†

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* 'Berichte d. deut. chem. Gesellsch.,' 1870, vol. 3, p. 68.

† See especially, Usher and Priestley, 'Roy. Soc. Proc.,' 1906, B, vol. 77, p. 369, where references to other papers are given.

Acting on the hypothesis outlined above, experiments with a view to testing the synthetic action of sunlight in presence of inorganic colloids were commenced by us in November, 1911, but for over a year only negative results were obtained, on account of difficulties in adjusting proper concentrations of solution, securing adequate illumination in vessels made of suitable material, and obtaining delicate enough methods for separating and identifying formaldehyde. After overcoming these experimental difficulties, and with the aid of the brighter sunshine, a large number of positive results have been obtained, within the past few weeks, and the synthesis of formaldehyde under the conditions to be described below has been shown quite unmistakably by all the usual colour-reactions for formaldehyde.

It has also been shown that it is the ultra-violet rays which are most effective. The use of quartz flasks as containers for the colloid gives, with the same intensity of insolation, definite results in a much shorter period, and lately we have found that a "Uviol" mercury arc, in a "Uviol" glass protecting vessel, immersed in an outer wider cylinder of glass so as to produce a thin layer of the colloid, through which the carbon dioxide is passed, gives quite clearly positive reactions for formaldehyde by all the colour tests, with an exposure to light of only four or five hours.

The results are obtained either with colloidal hydrated ferric oxide, or colloidal oxide of uranium, in exceedingly dilute solution. For example, in the case of uranium, 0·028 per cent. of the oxide, and, in the case of the iron, 0·113 per cent. of ferric oxide were the concentrations used.

Controls carried out in the absence of light gave no formaldehyde, and all precautions were taken to exclude contamination in any way. The same point is shown by our earlier unsuccessful experiments in which the illumination was inadequate.

Formaldehyde in small amounts has already been synthesised from inorganic sources in several reactions in which hydrogen in the nascent condition, or adsorbed in palladium, has been present. But there is here chemical energy as such, presented by the hydrogen, and so far as we are aware there is no case known of utilisation of light energy by an inorganic catalyst which does not itself become altered in the reaction.

Thus Bach* obtained formaldehyde from hydrogen-palladium and carbon dioxide, Fenton† obtained it by the action of carbon dioxide and water on metallic magnesium, and other observers have obtained it by the interaction of dilute sodium amalgam and moist carbon dioxide.

The only experiment approaching more closely to our own as recorded below is one devised by Bach,* and later repeated with modifications by Euler† and by Usher and Priestley.‡

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* 'Comptes Rendus,' 1898, vol. 126, p. 79.

† 'Journ. Chem. Soc. Trans.,' 1907, vol. 91, p. 687.

Bach passed a current of carbon dioxide through a solution of 1·5 per cent. *crystalloidal* uranium acetate, and in the presence of light obtained a precipitate of mixed oxides of uranium, which did not occur when light was excluded during the passage of the carbon dioxide. He adduces no direct experimental evidence of the presence of formaldehyde in the solution, but makes the hypothesis that the carbonic acid by the action of light forms formaldehyde and per-carbonic acid. Then the per-carbonic acid decomposes and forms peroxide of uranium. Lastly, the formaldehyde attacks uranic oxide forming lower oxides recognisable by their colour in the precipitate of mixed oxide. In confirmation he shows that when peroxide of uranium and actually added formaldehyde are exposed to sunlight a reduction to lower oxide occurs with production first of a green and then a violet colour.

It will be observed, first that the crystalloidal salt of uranium employed undergoes permanent change, and secondly that there is no clear evidence of formation of formaldehyde, although our experiments recorded below show that Bach's hypothesis is probably a correct one. All the experiment actually proves, however, is that acetate of uranium in crystalloidal solution, in presence of carbon dioxide and sunlight, is decomposed, yielding a mixture of oxides of uranium. At the same time it is an important pioneer experiment in this field.

In a later experiment, Bach obtained evidence of the formation of formaldehyde by exposing to light a solution of dimethylaniline in dilute sulphuric acid through which carbon dioxide was passed. There is here, however, the objection of using an organic body as catalyst, although the reaction is interesting as a photo-synthesis.

The same objection holds, as to the presence of the acetic acid anion in the uranium acetate solution used above, since this might serve as a source of formaldehyde.

A considerable increase in our knowledge was given in the papers of Usher and Priestley quoted above, in which they repeated and extended Bach's experiment.

The experiments of Bach were repeated and confirmed by these authors, both as to the production of peroxide and formaldehyde. The amount of decomposition obtained in three weeks in bright weather was extremely small, and this was ascribed to the poorness of the uranium as a catalyst, and the non-removal of the separated oxygen which remained as uranium peroxide and acted as a destructive agent upon the formaldehyde.

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* 'Comptes Rendus,' 1893, vol. 116, p. 1145.

† 'Berichte d. deut. chem. Gesellsch.,' 1904, Jahrgang 37, vol. 2, p. 3411.

‡ 'Roy. Soc. Proc.,' 1906, B, vol. 77, p. 369 ; and 1906, B, vol. 78, p. 322.

The authors accordingly took tubes of Jena glass, cooled them in liquid air, passed in carbon dioxide, sealed, and exposed for 24 hours, suspended outside a south window, in bright sunlight. A precipitate appeared after 15 minutes' exposure, and in 24 hours the reaction was complete. The tubes on opening were found to contain uranium peroxide and formic acid, but no formaldehyde.

Usher and Priestley then repeated their experiments using uranium sulphate instead of the acetate. Carbon dioxide was passed through a 2-per-cent. crystalloidal uranium sulphate solution, and the solution was exposed to sunlight on a roof for nearly a fortnight. Several grammes of a precipitate of mixed oxides of uranium were obtained of a pale violet colour. The greater part was a mixture of uranous and ordinary uranic hydroxides soluble in acetic acid. The insoluble residue was a hydrate of uranium peroxide. The filtrate from all these mixed hydroxides was distilled and examined for formaldehyde. None, however, was found, though the liquid reduced Fehling's solution and silver nitrate. It was subsequently found to contain formic acid, the lead salt of which was prepared and identified. The undistilled residue was then evaporated down, and when nearly solid was repeatedly extracted with dry ether in order to remove any formic acid which had not evaporated. The residual solid was extracted with absolute alcohol, and the solution on evaporation left a small quantity of a brown syrup, bitter to the taste, which reduced Fehling's solution. It could not be proven that this formed an osazone, but it closely resembled in its properties a substance called "methylenitan" obtained by Butlerow from formaldehyde and milk of lime. The body was obtained in minute amount only.

This experiment constitutes a distinct advance, since the organic substances (formic acid and the body above mentioned) were obtained by the action of light on purely inorganic substances.

The points still left against the results, from the aspect mentioned at the outset of the present paper, are that the catalyst is an extremely rare one in nature, that it was used in high concentration in crystalloidal solution, and that it underwent changes in itself and was precipitated as the result of the reaction. The fact that formic acid was obtained instead of formaldehyde, looked at from our point of view, is relatively unimportant, since both are organic bodies of increased energy content.

By the use of many times more dilute colloidal uranic hydroxide, we have been able now to obtain formaldehyde, and this without precipitation or other visible change in our catalyst. Compared with a stronger solution of crystalloidal uranium nitrate alongside, and given the same exposure and general conditions, we have been able to show that the colloidal condition is much more active in this photo-chemical reaction. In this comparative experiment the crystalloidal uranium nitrate was precipitated while no precipitation whatever occurred in the colloidal uranic hydroxide.

Passing on, we have shown that similar photo-synthesis of organic from inorganic matter easily occurs with colloidal ferric hydroxide, and by the use of the "Uviol" mercury lamp we are enabled to experiment at all times and obtain synthetic results readily in a few hours, a consideration of some importance in working in a country where sunlight is so variable, and so often unavailable for days.

The experiments with uranic hydroxide and ferric hydroxide were made concurrently in time, but for convenience we shall describe first the uranium experiments and then those with the ferric hydroxide.

Photo-synthesis by Colloidal Uranic Hydroxide.

Method of Preparation of the Colloidal Solution.—A strong solution of uranium nitrate (approximately 10 per cent.) is taken and treated in the cold by adding a saturated solution of ammonium carbonate until the precipitate just ceases to re-dissolve. The solution is then filtered and dialysed in a tube of parchment paper for several days against running water. The greater part of the uranium is still in the crystalloidal state, and dialyses away at this stage. Great care is required in the first step to get the proper relative amount of ammonium carbonate; also it is as well not to wait for the removal of the last traces of crystalloidal uranium, but to take the solution when it still gives a faint reaction with potassium ferrocyanide. The amount so left is, however, very minimal compared with the crystalloidal uranium solutions as used in the experiments recorded below, and nearly all the uranium at the end is present as colloidal uranic hydroxide.

This method is practically the same as that described by Graham for the preparation of colloidal ferric hydroxide and as used in the preparation of our colloidal iron solutions.

The amount of uranium in this solution was determined by evaporating a measured volume to dryness and weighing the residue, and was found to be 0·478 per cent. of Ur_2O_3.

In carrying out the photo-synthetic experiments this solution was either diluted 15- to 20-fold with distilled water and carbon dioxide, evolved from marble and pure hydrochloric acid and washed by water in a wash-bottle, passed through in a slow stream, or it was diluted to the same degree with distilled water previously saturated with carbon dioxide and sealed up hermetically in glass tubes, which were then exposed to such direct sunlight as was available on the flat roof of the laboratory.

It will accordingly be observed that the concentration of the colloidal uranic hydroxide in the solutions actually exposed to the light was only 0·024 to 0·035 per cent. The stock solution of the colloid had a pale lemon yellow colour, and the diluted solutions were almost colourless, showing just the merest trace of yellow colour, and the solutions throughout remained water clear. The colloidal solution is very sensitive, and is precipitated by traces of added crystalloid.

The test solution for aldehyde used in our earlier experiments was Schiff's reagent, which consists of a dilute solution of fuchsin (about 1 per cent. in water) through which sulphur dioxide is passed until it is just decolorised. This reagent added in the proportion of 3 drops to 10 c.c. of a dilute formaldehyde solution shows the presence of the latter by a distinct pink coloration, developing in about half an hour at a dilution of 1 in 500,000.

At a later stage our observations were confirmed by the Mulliken and Schryver tests.

Mulliken's test, as used by us, consists in adding two drops of a 3-per-cent. solution of gallic acid in absolute alcohol to 6 c.c. of strong sulphuric acid in a test-tube, and then pouring on the surface of this mixture 2 c.c. of the solution suspected to contain formaldehyde, when a light blue coloured ring develops at the junction and gradually penetrates the sulphuric acid.

Schryver's test was carried out by preparing a 5-per-cent. solution of potassium ferricyanide and a 1-per-cent. solution of phenylhydrazine hydrochloride which was used as follows:—To 10 c.c. of the solution suspected of containing formaldehyde 2 c.c. of the phenylhydrazine solution is added, then 1 c.c. of the potassium ferricyanide solution followed by 3 c.c. of concentrated hydrochloric acid, when a pink coloration appears. If this is diluted with water it becomes colourless, this colourless solution is shaken with ether, and the ethereal solution which is not coloured is separated off. Finally the ethereal solution is shaken up with a few cubic centimetres of concentrated hydrochloric acid, when, if formaldehyde be present, a strong pink or carmine colour appears in the hydrochloric acid layer.

All three of these colour tests for formaldehyde have been obtained on many occasions.

Experiment I.—20 c.c. of dilute colloidal uranic hydroxide solution containing approximately 0·03 per cent. of Ur_2O_3 was placed in a test-tube on the roof, and carbon dioxide generated in a Kipp, and washed as above described, was slowly bubbled through. The day was a bright sunny one (May 26,

1913) and the experiment was continued all day. Half of the solution was taken out, and of this the greater part was distilled off from the colloid. Tested with Schiff's reagent this distillate gave a distinct pink coloration within half an hour. The remaining half of the colloidal solution was kept on the following day, which was also a bright sunny day, on the roof with carbon dioxide bubbling slowly through it, it was then tested in the same way for aldehyde, and gave a still stronger reaction. Blanks were carried out with distilled water and the Schiff's reagent and the contrast was most marked, the distillate from the colloid exposed to light giving a deep pink, while the distilled water control remained colourless.

Experiment II.—Thirty c.c. of the same dilute colloidal uranium solution was taken in a wide test-tube, saturated with carbon dioxide by bubbling the gas through, and then hermetically sealed. The tube was left resting on its side on the roof for two days, both of which had a good deal of bright sunshine. The contents of the tube showed no visible alteration or precipitation at the end of the period. The tube was now opened and about two-thirds of its contents were distilled off. The distillate tested with Schiff's reagent gives a marked formaldehyde reaction.

Experiment III.—A soda-water syphon with a "sparklet" apparatus for charging with CO_2 was taken, and in it were placed 500 c.c. of distilled water, 30 c.c. of the stock colloidal uranium hydroxide solution, giving accordingly a concentration of 0·035 per cent. of Ur_2O_3.

This was charged with carbon dioxide from a "sparklet" bulb, and left on the roof for 13 days, of which 7 were bright days with strong sunlight. At the end of the period a portion was withdrawn and distilled. It gave strong positive reactions for formaldehyde with both Schiff's reagent and Schryver's test.

The stock solution of colloidal uranium hydroxide diluted equally and distilled without previous exposure to light gave negative results in both tests.

Experiment IV. Contrast of Action of Colloidal Uranium and Crystalloidal Uranium.—Two glass tubes were taken of similar dimensions; in the first were placed 20 c.c. of distilled water saturated with carbon dioxide from a "sparklet" syphon, and 2 c.c. of stock colloidal uranium hydroxide solution; in the other tube, 20 c.c. of the water charged with carbon dioxide from the same syphon and 0·2 c.c. of 20-per-cent. crystalloidal uranium nitrate solution. At the end of the experiment, by drying and incinerating a measured volume, the percentage of uranic oxide was determined, and it was found that the colloidal solution contained 0·041 per cent., and the crystalloidal 0·08 per cent., so that the crystalloidal solution was approximately double in concentration that of the colloidal. The two tubes were hermetically sealed and placed on the roof for six days, three of which had bright sunshine, the others very cloudy and raining. The two tubes were opened, and the contents separately distilled in a similar fashion. The distillate from the crystalloid showed negative results with the Schiff s test. while the distillate from the colloid gave a most strongly marked positive reaction.

Experiment V. Illustrating the Necessity for Strong Direct Sunlight.—Two solutions, one of colloidal uranic oxide, the other crystalloidal uranium nitrate of approximately equal concentration, were taken, of each 50 c.c., in a glass tube, and washed carbon dioxide was bubbled through each in a slow stream. These were exposed on the roof for two days. Both these days were dull with practically no sunshine; there was, however, fairly bright diffuse daylight. The contents were then distilled as in the preceding experiments, but negative results were obtained in both cases.

Experiment VI.—Four similar wide glass tubes were taken, and into each was introduced 30 c.c. of distilled water charged previously with carbon dioxide, and 2 c.c. of colloidal uranic oxide solution, containing 0·478 per cent. of Ur_2O_3. Accordingly, the concentration of colloid in each case was approximately 0·03 per cent., or 3 in 10,000 parts. The four glass tubes were then sealed up hermetically and treated as follows:—

1. The first tube was exposed on the roof to such sunlight as was available for six days, in four of which there was brilliant sunshine all day.

2. The second tube was preserved for the same period in a dark cupboard in the laboratory.

3. The third tube was immersed in a wider open glass tube containing a strong alcoholic solution of chlorophyll, so as to give a chlorophyll shade all round it, between it and the sunlight, and then the open tube was closed by a cork. This tube was then, thus sheathed, exposed to the sunlight on the roof alongside the first tube and for a similar period.

4. The fourth tube was immersed in a 5-per-cent. solution of quinine sulphate in a similar manner to that described for tube No. 3, and was then exposed in like manner to Nos. 1 and 3, and for the same period.

The contents of the four tubes were distilled off in each case, and the four distillates were comparatively tested alongside one another, using the Schiff's and Mulliken's tests.

The tube kept in the dark gave completely negative results, while all three exposed to the light (Nos. 1, 3, and 4) gave positive results; the tube surrounded by chlorophyll (No. 3) was the strongest, and next was that surrounded by the quinine solution, both being more marked than the tube exposed to direct sunlight. But much more experimentation is required here. There is, however, no doubt that active rays penetrate both chlorophyll and quinine solutions, as the reactions were most distinct, and it appeared as if these solutions possibly had prevented the passage of rays with a slowing effect on the reaction.

Experiment VII. Dilute Colloidal Uranic Oxide Solutions Exposed to Light from a " Uviol" Mercury Arc in a Transparent Quartz Test-tube.—40 c.c. of a colloidal uranic oxide solution made by diluting the stock solution 15-fold, and so containing 0·03 per cent. of the colloid, were placed in a tube of transparent quartz, and after saturation with washed carbon dioxide from a Kipp apparatus were exposed about 2½ inches from a " Uviol " mercury arc for the period during three days in which the lamp was lit, probably about 12 hours in all. At the end of the period the fluid was distilled and the distillate tested by Schryver's test. It gave a strongly marked reaction, corresponding to at least 1 part in 100,000 of formaldehyde.

Photo-synthesis by Colloidal Ferric Hydroxide.

Preparation of Colloidal Solution.—The colloidal ferric hydroxide solution was prepared after the method originally given by Graham.* A strong solution of ferric chloride is taken, about 20 per cent., and to this a saturated solution of ammonium carbonate is added gradually with shaking so long as the precipitate first formed continues to dissolve. The solution is then dialysed for some days, 10 days or more, until the reaction for chloride becomes very faint. The solution so obtained is of a deep reddish brown colour, even in a 1-per-cent. solution, and if converted back into the crystalloidal form by boiling with a drop or two of acid the change is remarkable, to a pale lemon yellow colour. At the dilutions used in the experiments below the colour scarcely shows when reduced to the crystalloidal condition, but in the colloidal condition, even at this dilution, the solutions possess a deep sherry colour. The dilute solution in an ordinary small test-tube absorbs the blue of the spectrum completely, as shown by a pocket spectroscope. When viewed by light transmitted from a " Uviol " mercury lamp, the solution viewed directly transmits a yellowish green light, and at the sides, reflected from the glass surfaces, there is a deep green fluorescence, which reminds one strongly of the fluorescence of a strong solution of chlorophyll.

The colloidal iron solution so prepared is readily coagulated by boiling, and is most sensitive to added crystalloids; it is thrown out by 1 part in

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* 'Phil. Trans.,' 1861, vol. 151, p. 208.

1000 of ammonium carbonate, and a mere trace of deci-normal caustic soda throws it completely down. It is in a delicately reactive metastable condition, which reminds one forcibly, as it did Graham 50 years ago, of the proteins and the constituents of living cells.

When set up in the "Uviol" apparatus and the transmitted light observed with a spectroscope, it is seen that the bright lines of the mercury arc spectrum in the blue and violet have entirely disappeared, and the only ones now visible are those of the red, orange, and green. An examination of the solar spectrum shows complete absorption of all higher wave-lengths than green.

There is this difference between the solar and the mercury arc light absorption, that in the former there is a continuous spectrum absorbed from green onward, while in the mercury arc spectrum the absorption is that of three sets of wave-lengths, one at the junction of green and blue, the other far over in the blue, and the third in the violet portion of the visible spectrum. We have not hitherto been able to observe the absorption of the ultra-visible rays. The light energy from these definite wave-lengths of the spectrum seems, however, from the results recorded below, to be very effective for the particular synthesis under consideration.

In describing the synthetic results with the ferric oxide colloid, a few earlier experiments made in glass vessels with rather poor daylight illumination may be passed over, merely remarking that these led us on to the others in which unmistakable evidence of organic synthesis was obtained, and only the latter are here recorded. With sufficient illumination either with sunlight or the mercury arc spectrum, and especially when "Uviol" glass or quartz has been used, we have never failed to obtain clear evidence of synthesis.

Experiment I.—A dilute solution of colloidal ferric hydroxide containing 0·2 per cent. of Fe_2O_3, was placed in a thin blown flat-sided glass bottle, made like a wash-bottle with ground glass stoppers. A slow current of carbon dioxide, washed by passing through a wash-bottle containing water, after evolution from marble in a Kipp apparatus, was passed through the colloidal solution during two days of fairly bright sunshine on the laboratory roof. On distillation this gave a moderately strong positive reaction to the Schiff's test.

Experiment II.—A glass soda-water syphon with a "sparklet" apparatus attached was charged with 500 c.c. of distilled water and 5 c.c. of a colloidal ferric oxide solution, and after dilution contained about 0·05 per cent. of colloidal ferric oxide. This was saturated with carbon dioxide by a sparklet bulb, and left on the roof for a period of 20 days, in which there were about

13 days of bright sunshine. At the end of the period, 40 c.c. were withdrawn and distilled. A very distinct positive reaction for formaldehyde was obtained with Schryver's test.

Experiment III. Exposure to Sunlight in Transparent Silica Flask.—A colloidal solution of ferric hydroxide of a concentration of 0·14 per cent., measuring 50 c.c., was placed in a silica flask through which carbon dioxide was passed, on the roof. The experiment lasted for two days, of which the first was dull, and the second almost continuous bright sunshine. On distillation this gave a most marked positive reaction with Schiff's test, indicating from the short period of exposure that the reaction probably proceeds more rapidly in silica vessels which are more transparent to the shorter wave-lengths of light.

Experiment IV. Exposure to Mercury Arc Lamp with Uviol Glass Shade.—A colloidal solution of ferric hydroxide diluted 1 in 20 from a stock solution of 2·26 per cent., and hence containing 0·113 per cent. of colloid, was placed in a wide 1000 c.c. measuring cylinder. In this glass cylinder was immersed a wide round-bottomed tube of "Uviol" glass of somewhat smaller diameter to serve as a protector between the heated lamp to be placed in the interior, and the surrounding fluid between the "Uviol" tube and the outer cylinder of glass. The mercury lamp was then set going, and inserted in the centre of the "Uviol" glass protecting tube. The mercury lamp tube, also of "Uviol" glass, had a diameter of 3 cm. approximately, the protecting tube a diameter of 5·4 cm. externally, and the outer glass cylinder an internal diameter of about 6·2 cm. The light generated at a distance of about 2 cm., after passing two thicknesses of "Uviol" glass and a layer of air of somewhat less than a centimetre, passed through a layer of the colloidal solution about 4 to 5 mm. thick and then reached the outer glass vessel. On account of the rounded bottom of the "Uviol" protecting tube there was a certain considerable volume of the solution at the bottom not so well illuminated. The total volume of colloidal solution at the bottom and in the annular space between the two tubes was approximately 300 c.c.

A slow steady stream of washed carbon dioxide was passed through the colloidal solution.

After about half-an-hour's running the colloidal solution became too warm and began to coagulate, so the current was switched off and the lamp allowed to cool. The whole was then immersed in a wide square-sided glass jar of about 17 litres capacity through which a stream of cold water was continuously circulated. The lamp was now re-lit and kept going for a period of 2 hours, making 2½ hours of illumination in all. In this first experiment with the "Uviol" apparatus, the colloidal solution had coagulated at the end but this did not happen in later experiments when the heat was better regulated.

The colloidal solution was removed and tested for formaldehyde in two ways. One portion was distilled as in the previously recorded experiments and the distillate gave a most marked positive reaction with Schiff's reagent —a deep pink coloration. A second part was simply filtered off from the coagulated iron precipitate, and at once tested. It also gave a marked positive reaction both with Schiff's and Mulliken's tests.

Experiment V.—An experiment of the same type as the preceding one. A colloidal solution of 1 in 20 of the same stock solution was taken, and exposed to the mercury arc light as before. Illuminated during two periods of 55 minutes and 2 hours 50 minutes, respectively, making 3 hours 45 minutes in all. Part of the solution distilled off gives a distinct positive reaction with Schiff's test. Another portion coagulated by boiling, and filtered from iron, but not distilled, gives a positive reaction with gallic acid and concentrated sulphuric acid (Mulliken's test).

The experiment was continued next day from 10.15 A.M. till 2.15 P.M.; at the end of this 4 hours' period the solution coagulated, owing to a failure in the water circulation and the temperature rising. The coagulated fluid was filtered and the filtrate tested, it gave a marked positive effect with Schiff's test and also a positive result with Mulliken's test, a trace of iron left over interfered with the Schryver test, but on distillation this also was obtained.

Experiment VI.—This was conducted similarly to the above experiments IV and V for a period of 1 hour and 40 minutes. Then a trace of ammonium carbonate amounting to only 1 part in 1000 was added with the view of forming hexamethylenetetramin and so concentrating the formaldehyde, but even this trace coagulated the solution.

The experiment was, however, continued for 5½ hours additional. A small portion was acidified and distilled. The distillate gave a marked positive reaction with Schryver's test.

Experiment VII.—A dilute colloidal solution of ferric hydroxide (1 in 15) of stock solution, equivalent to 0·13 per cent. of ferric oxide, was exposed after saturation with carbon dioxide, in a silica test-tube 3 inches away from the mercury arc, during the time the lamp was lit on three days, probably about 12 hours in all. A most marked reaction to all tests. The reaction quantitatively is slightly less than the uranium effect, see Experiment VII of previous section, and throughout it appears that the uranium catalyst is somewhat more powerful than the ferric catalyst.

Conclusions.

Organic matter (aldehyde) has been synthesised from inorganic colloidal uranic and ferric hydroxides in very dilute solution. These colloids act as catalysts for light energy, converting it into chemical energy in a reduction process similar to the first stage of synthesis of organic from inorganic substances in the green plant by the agency of chlorophyll.

Such a synthesis occurring in nature probably forms the first step in the origin of life. For chlorophyll and protoplasm are substances of far too complex chemical constitution to be regarded as the first step in the evolution of the organic from the inorganic.

Without the presence of organic material, when life was arising in the world, any continuance of life would, however, be impossible.

The process of evolution of simple organic substances having once begun, as now experimentally demonstrated, substances of more and more complex organic nature would arise from these with additional uptake of energy. Later, organic colloids would be formed, possessing meta-stable properties, and these would begin to show the properties possessed by living matter of balanced equilibrium, and up-and-down energy transformations following variations in environment.

There can be little question that such energy changes as are above described occur at present, and are leading always to fresh evolutions of more complex organic substances, and so towards life, and equally is it true that they must occur on any planet containing the necessary elements for the evolution of inorganic colloids and exposed to light energy under suitable conditions of environment.

The Nature of the Coagulant of the Venom of Echis carinatus, *a Small Indian Viper.*

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(From the Liverpool Cancer Research Laboratory, the University, Liverpool.)

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The effect of intravascular injection of the venom of the Australian black snake (*Notechis porphyriacus*) upon blood coagulation was studied by C. J. Martin,* who, noticing the similarity of the resulting changes with those described by Wooldridge,† after intravascular injection of "tissue fibrinogen," inferred that in both cases the mechanism involved was the same, namely, the introduction of nucleo-proteins into the blood stream, the liberation of these substances after the injection of viper venom being presumably due to the latter damaging the endothelial cells of the blood-vessels.

Subsequently it was observed by Lamb‡ that the addition of viper venom to citrated or oxalated blood plasma also caused clotting. Martin§ therefore abandoned the explanation he had previously suggested and concluded that the coagulant action of viper venom was due to fibrin ferment.

The subject was later reinvestigated by J. Mellanby,|| who recognised that

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* C. J. Martin, "On some Effects upon the Blood produced by the Venom of the Australian Black Snake," 'Journ. Physiol.,' 1893, vol. 15, p. 380; "On the Physiological Action of the Venom of the Australian Black Snake," 'Roy. Soc. N.S.W. Proc.,' 1895; "Subcutaneous and Intravenous Injection of the Venoms of the Australian Snakes," 'Roy. Soc. N.S.W. Proc.,' 1896.

† L. C. Wooldridge, "On the Origin of the Fibrin Ferment," 'Roy. Soc. Proc.,' 1884, vol. 36, p. 417.

‡ G. Lamb, "On the Actions of Venoms of the Cobra (*Naja tripudians*) and of the Daboia (*Daboia russellii*) on the Red Blood Corpuscles and on the Blood Plasma," 'Scientific Memoirs by Officers of the Medical and Sanitary Department of the Government of India,' 1903, New Series, No. 4.

§ C. J. Martin, "Observations upon Fibrin Ferments in the Venoms of Snakes and the Time Relations of their Action," 'Journ. Physiol.,' 1905, vol. 32, p. 207.

|| J. Mellanby, "The Coagulation of the Blood, Part II," 'Journ. Physiol.,' 1909, vol. 38, p. 441.

the nucleo-protein invoked in the earlier hypothesis of C. J. Martin would, in more modern terminology, be called thrombokinase. Mellanby concluded from his observations that viper venoms did not contain fibrin ferment, but that the active blood-coagulating agent contained in these venoms consisted of pure thrombokinase. He based this conclusion upon the circumstance that (1) the coagulant effect of viper venom upon fibrinogen is increased by the addition of calcium chloride, and (2) that when venom is allowed to act upon prothrombin (in the absence of added calcium chloride or in presence of potassium oxalate) fibrin ferment is produced. As regards (1) it may be pointed out that, as shown by Bordet and Delange,* the action of fibrin ferment upon fluid blood plasma is increased in the presence of calcium chloride.

With a view of throwing additional light upon the problem whether the coagulant action of viper venom is due to thrombin or thrombokinase, it was determined to investigate further the action of heated and unheated venom upon circulating blood plasma. Before proceeding to describe the experiments made with viper venom it will, however, be necessary to consider the action of heat upon thrombin and thrombokinase respectively, and also to refer to the effect, in respect of production of fibrin, of the injection of these substances into the blood stream.

In order to obtain a solution of thrombin fibrinogen was coagulated by a small amount of thrombokinase in presence of calcium chloride; the fluid expressed from the coagulum furnished a supply of thrombin. As source of thrombokinase red-cell stromata were used in most experiments; less frequently peptone was employed.

The venom employed was that of the phoorsa, *Echis carinatus.* For a supply of this venom I am indebted to the great kindness of Dr. C. J. Martin, to whom my best thanks are due for his generous gift.

Action of Thrombin and Thrombokinase upon Circulating Blood Plasma.

The coagulant effect of thrombin and thrombokinase is usually tested *in vitro.* For this purpose a solution of fibrinogen is generally employed; less frequently fluid blood plasma is used. It has, however, been pointed out in a previous communication† that the action of blood coagulants may be studied more conveniently by intravascular injection, for if, after injection, separation of fibrin occurs, this can be recognised by the presence of masses of fibrin in the blood-vessels, particularly of the lungs. This method has been adopted in the present investigation.

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* J. Bordet and L. Delange, "La Coagulation du Sang et la Genèse de la Thrombine," 'Annales de l'Institut Pasteur,' 1912, vol. 26, p. 657.

† J. O. Wakelin Barratt, "On Fibrinæmia," 'Journ. Pathol. and Bacteriol.,' 1913, vol. 17, p. 303.

The injection of a solution of thrombin was carried out after the manner of the following experiment:—

Experiment 1.—A solution of fibrinogen measuring 16 c.c. was prepared, as described in the next section, from 16 c.c. of rabbit's blood plasma. To this 1·2 c.c. of a suspension of the red-cell stromata of the rabbit* was added, together with 1·2 c.c. of 0·6-per-cent. calcium chloride solution. A firm clot was formed at the end of 30 minutes at 37° C. After the lapse of a further period of 60 minutes 17 c.c. of fluid was expressed from the clot by means of a glass rod; this was injected into the vein of the ear of a rabbit weighing 850 grm. At the end of injection, which occupied nine minutes, the animal became convulsed and died four minutes after the completion of injection. A *post-mortem* examination was made without delay: the blood in the heart and great blood-vessels was fluid; the coagulation time of blood taken from the heart was two minutes; 4 c.c. of blood obtained on opening the heart coagulated slowly at room temperature, forming a moderately firm clot, which had retracted to about the normal extent next morning; the lungs collapsed rapidly upon opening the chest.

When sections of the lung are made after intravenous injection of thrombin, fibrin masses are found in the blood-vessels in amount depending upon the quantity of thrombin injected, but in no case are fibrin masses observed in more than a small number of the blood vessels. The appearance of the fibrin, which is illustrated elsewhere,† varies considerably, being obviously in part determined by the time elapsing between injection and death. Sometimes, though rarely, a very fine network is seen, resembling that observed when a section is made of a clot formed in a solution of fibrinogen after the addition of thrombin, or of thrombokinase and calcium chloride, as in the experiments recorded in the next section. More usually an irregular coarse network is seen, resulting from the contraction or compression in the blood stream of the delicate fibrils first formed, the process being comparable to that occurring when blood is whipped. Frequently the change is carried a step further and dense masses of fibrin of varying size are seen, sometimes forming irregular plugs partly filling the arterioles, sometimes in the form of threads of varying length in the capillaries. It is found that after the injection of large quantities of thrombin the amount of fibrin obtainable when blood collected from the heart is allowed to coagulate is markedly reduced in amount, sometimes the blood remaining permanently liquid;[*] in such cases the quantity of fibrin in the blood vessels of the lungs is conspicuous. If, however, the amount of thrombin injected is small some difficulty may be experienced in finding fibrin in the pulmonary vessels, even though symptoms of illness, generally not severe, have followed injection. In the viscera, other than the lungs, fibrin is less readily observed and not infrequently appears to be absent.

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* *Cp.* Experiment 2, p. 180.

† J. O. Wakelin Barratt, *loc. cit.*

When the coagulant action of thrombin *in vitro* is compared with that following upon its injection into the blood stream, it is obvious that the process is essentially the same in both cases, such differences as appear being due to the churning action of the circulatory movement of the blood causing condensation of the delicate fibrin fibrils first formed, and thus leading to the appearance of solid masses of fibrin of varying size.

The injection of thrombokinase, contained in a suspension of the red-cell stromata of the rabbit,[†] was effected in a series of observations carried out after the following method:—

Experiment 2.—Oxalated rabbit's blood was centrifugalised until the red cells were completely precipitated. After pipetting off the supernatant plasma the red cells were twice washed with 0·85-per-cent. sodium chloride solution, and were then laked by the addition of an equal volume of distilled water. Solid sodium chloride was then added in amount necessary to form 0·85 per cent. of the distilled water employed and the liquid again centrifugalised, whereby the red-cell stromata were precipitated. After pipetting off the supernatant solution of hæmoglobin 0·85-per-cent. sodium chloride solution was added in the amount required to make up the original volume. The resulting liquid, which contained the red-cell stromata, together with approximately half the amount of hæmoglobin present before laking,[‡] served as source of thrombokinase. Of this liquid 5 c.c. were now injected in the course of three minutes into the vein of the ear of a rabbit weighing 1005 grm. At the end of injection the animal appeared to be unaffected. Shortly afterwards, however, it became convulsed, respiration failed, and death occurred at the end of 10 minutes. On opening the chest immediately after death the lungs were observed to collapse rapidly; blood obtained from the heart clotted slowly at room temperature; no clots were found in the heart and great blood-vessels.

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[*] When this is observed, the addition of thrombin fails to produce clotting *in vitro*.

[†] The coagulant action of a suspension of red-cell stromata upon fibrinogen *in vitro* is exhibited in the next section (p. 185).

[‡] More hæmoglobin may be removed from the stromata by repeated washing with 0·85-per-cent. sodium chloride. If this is done, larger amounts of stromata may require to be injected in order to cause death. The dissolved hæmoglobin does not appear to give rise to any recognisable ill effect. *Cp.* J. O. Wakelin Barratt and W. Yorke, "Ueber Hämoglobinämie," 'Zeitschr. f. Immunitätsforschung u. exp. Therapie,' 1912, vol. 12, p. 333.

On microscopical examination of sections of the viscera of rabbits which had received by intravenous injection lethal amounts of red-cell stromata, fibrin masses were sometimes, but not invariably, found in the blood-vessels of the lungs, being present in very sparse numbers, even though relatively large quantities of stromata (*i.e.*, as much as is obtainable from 4 c.c. to 12 c.c. of rabbit's red cells per kilogramme of body weight of the animal injected) were introduced. It is to be noted also that no obviously defective formation of fibrin in blood obtained from the heart could be observed, clotting generally resulting in the formation of a firm coagulum retracting normally in the course of 24 hours; occasionally, however, the clot formed was distinctly soft. Coagulation was usually delayed, the period of onset of coagulation at 37° C. being one to six minutes.*

The intravascular injection of thrombokinase in the form of peptone was carried out in a series of experiments of which the following is an example:—

Experiment 3.—Into the vein of the ear of a rabbit weighing 542 grm., 24 c.c. of a 9-per-cent. solution of Witte's peptone† (the solution was observed to be alkaline to litmus paper) was injected at the rate of 2 c.c. per minute; 10 minutes after the completion of injection 5 c.c. of blood were removed from the internal carotid artery; the blood remained fluid at the end of 50 minutes at room temperature (17° C.), but at the end of 70 minutes a soft clot had formed which after 2 hours had become firm and was found to be considerably retracted; 2 c.c. of blood removed from the vein of the ear shortly before injection formed a firm clot at the end of 12 minutes.

On microscopical examination of the viscera of rabbits killed 5 to 30 minutes after intravenous injection of a solution of peptone no fibrin masses were found in the blood-vessels of the lungs or elsewhere. No obvious defect of fibrinogen was observable after the injection of peptone, the blood clotting slowly, but after a time forming a firm coagulum, which retracted to about the normal extent. The amount of peptone (which it should be noted was not prepared from the flesh of the rabbit) injected in these experiments ranged from 0·3 to 7·3 grm. per kilogramme of body weight. Blood obtained from the internal carotid artery 10 to 20 minutes after injection was in no case permanently liquid, though as already mentioned coagulation was delayed, the onset occurring at room temperature in 25 to 60 minutes, the normal period being about 15 minutes.

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* J. O. W. Barratt and W. Yorke, *loc. cit.*

† This amount corresponds to 4 grm. of peptone per kilogramme body weight.

The effect of peptone upon the coagulability of the blood was further studied in rabbits in a series of experiments in which blood was withdrawn by means of a paraffined cannula from the internal carotid artery, about 5 minutes after intravenous injection of peptone, and received into: (1) a paraffined tube; (2) a second paraffined tube; and (3) a glass tube not paraffined. Immediately before injection blood was collected from the internal carotid artery into (4) a glass tube not paraffined. Tube 1 was centrifugalised and liquid plasma ($1a$) obtained, free from blood cells, which was preserved in a paraffined tube. In this way it was found possible to compare the time of onset of coagulation under different experimental conditions. Thus in one experiment, which may be taken as a type, a firm clot formed in (4) at the end of 10 minutes; a soft clot formed in (3) at the end of 25 minutes (remaining still soft at the end of 3½ hours); the blood in (2) formed a soft clot at the end of two hours, still remaining soft and not retracted at the end of two days; the plasma in ($1a$) remained perfectly liquid at room temperature (15° to 17° C.) for four days, changed to a somewhat thick liquid at the end of five days and had formed a soft clot at the end of seven days. Experiments of this kind cannot be satisfactorily carried out if instead of peptone red cell stromata are employed, owing to the exceedingly lethal character of such injections. A similar defect of coagulability may be observed when peptone acts upon a solution of fibrinogen *in vitro*; thus in the series of tests given on p. 186 a fairly firm clot formed in the second tube, while in the next three tubes the clot became increasingly soft, being exceedingly so in the fifth tube; nevertheless at the end of two days all these tubes contained clots of normal firmness.*

It is clear from the above that the effect of intravenous injection of thrombokinase is essentially different from that of injection of thrombin. In the latter case an abundant intravascular formation of fibrin takes place attended with reduction of the amount of fibrinogen contained in the circulating fluid, while after the injection of thrombokinase in amounts equivalent, as far as coagulant action upon fibrinogen *in vitro* is concerned, to the thrombin injected,* little or no fibrinogen is removed, the amount of fibrin subsequently obtainable from the blood not being markedly diminished, so that it may be doubted whether the exceedingly limited separation of fibrin occasionally observed after injection of red cell stromata is more than a subsidiary and perhaps relatively unimportant process attending the action of thrombokinase; the difference, in respect of fibrin formation, between the injection of thrombin and thrombokinase cannot be attributed to the latter being introduced in a relatively insufficient amount.

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* The effect of injection of peptone into a dog, under conditions similar to those obtaining in Experiment 3, differs somewhat from that observed when a rabbit is employed. If the dog employed for experiment is resistant, the same delay of coagulation is observed, but the clot formed is much softer and retraction of the clot is imperfect or absent. If, however, a dog which is susceptible to the action of peptone is kept fasting for 48 hours before injection of peptone, then blood collected from the internal carotid artery remains, as is well known, for a long time liquid, though containing fibrinogen in apparently undiminished amount and clotting on the addition of thrombin.

The effect of thrombokinase upon circulating blood plasma is thus seen to be strikingly different from its action upon a solution of fibrinogen *in vitro* in presence of calcium chloride. In the latter case coagulation occurs; in the former no coagulant action is observed or at most only an insignificant quantity of fibrinogen is converted into fibrin. The two experiments are, however, essentially different. When thrombokinase acts *in vitro* its action is directed to the conversion of prothrombin into thrombin in presence of calcium chloride. When on the other hand thrombokinase acts upon circulating blood plasma in presence of the free calcium salt contained in the plasma (and the same is true if the calcium content of the plasma is increased by injection of calcium chloride) the non-production of thrombin proves that prothrombin is not present in the circulating blood plasma. By a similar mode of reasoning based upon experiments *in vitro*, Bordet and Delange† have shown that prothrombin does not at first exist in fluid plasma (*plasma limpide*), whence these observers conclude that in this fluid it is represented by an antecedent body which bears the same relation to prothrombin (serozyme of Bordet) that prothrombin bears to thrombin; and as the term fibrin-ferment is applied to thrombin, which reacts with fibrinogen to form fibrin, so, using the same mode of nomenclature, the substance which takes part in the conversion of proserozyme into serozyme may be termed serozyme-ferment, though the exact mode in which the latter substance is concerned in the coagulation of shed blood has still to be determined. The explanation of the failure of thrombokinase when injected into the blood stream to cause fibrin formation lies, therefore, in the fact that it is unable of itself to change proserozyme into serozyme. In those experiments in which, after the injection of red-cell stromata, a small amount of fibrin is recognisable in the blood-vessels of the lungs, it would appear that either a small amount of thrombin or else of serozyme-ferment is also introduced or set free at the time of injection, leading to the intravascular separation of fibrin in relatively insignificant quantity.

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* If the amounts injected per kilogramme of body weight in Experiments 1, 2, and 3 had been such as would coagulate *in vitro* equal quantities of fibrinogen solution, according to the data given in the next section, then the amount of stromata injected in Experiment 2 would have been increased two and a half times, while the amount of peptone injected in Experiment 3 would have been reduced to one-twelfth.

† J. Bordet and L. Delange, *loc. cit.*

It is obvious that the above method of injection into the circulating blood plasma will enable a liquid, which has been shown by tests *in vitro* to contain thrombin, to be distinguished from a second liquid which has similarly been shown to contain thrombokinase. The plasma contained in circulating blood is, moreover, the only liquid to which the designation "fluid plasma" or "stable plasma" can with propriety be applied. Up to the present no "fluid plasma" has been prepared which is not recognisably different from circulating blood plasma.

Effect of Heat upon Thrombin and Thrombokinase.

Method.—A solution of fibrinogen was obtained as follows from plasma prepared by Freund's method.* The internal carotid artery of a rabbit was exposed and ligatured in its upper part, a spring forceps being applied to the lower part of the vessel, which was then opened below the ligature and thoroughly washed out with 0·85-per-cent. solution of sodium chloride by means of a paraffined capillary pipette, after which a paraffined cannula was introduced. By releasing the forceps blood was allowed to flow from the cannula into a paraffined tube, which was then centrifugalised until the red cells were precipitated. The supernatant liquid plasma was next removed with a paraffined pipette and mixed with 20 volumes of distilled water through which a stream of CO_2 had been passed. The fibrinogen, which was thereby precipitated from solution, was collected by centrifugalisation and dissolved in 0·85-per-cent. solution of sodium chloride, the volume of the liquid thus obtained being made equal to that of the plasma employed.

A solution of thrombin was prepared by adding to 10 c.c. of solution of fibrinogen 0·9 c.c. of a 0·6-per-cent. solution of calcium chloride, together with such an amount of a suspension of stromata of the red cells of the rabbit† (usually about 2·5 c.c.) as would produce a coagulum at the end of 15 minutes at 37° C. From this coagulum about 13 c.c. of fluid (= thrombin solution) was expressed with a glass rod at the end of two hours.

Thrombin is destroyed by exposure to a temperature of 55° C. for 30 minutes, or to a temperature of 60° C. for about five minutes. This effect of heating is illustrated below:—

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* E. Freund, "Ein Beitrag zur Kenntniss der Blutgerinnung," 'Wien med. Jahrbücher, 1886, p. 46; "Über die Ursache der Blutgerinnung," *ibid.*, 1888, p. 260.

† Prepared as described in the preceding section, p. 180.

Fibrinogen solution.	Thrombin solution.	0·6-per-cent. $CaCl_2$ solution.	0·85-per-cent. NaCl solution.	0·83-per-cent. solution of potassium oxalate.	Coagulation time (30° C.).
c.c.	c.c.	c.c.	c.c.	c.c.	
0·2	—	0·03	0·27	—	90 minutes.
0·2	0·01	0·03	0·26	—	4 ,,
0·2	0·03	0·03	0·24	—	3 ,,
0·2	0·10	0·03	0·17	—	45 seconds.
0·2	0·30*	0·03	0·07	—	18 minutes.
0·2	—	—	0·27	0·03	3 hours.
0·2	0·01	—	0·27	0·03	4 minutes.
0·2	0·03	—	0·26	0·03	3 ,,
0·2	0·10	—	0·24	0·03	45 seconds.
0·2	0·30*	—	0·07	0·03	18 minutes.

* Previously heated to 60° for 5 minutes.

The fibrinogen solution at 30° C. formed a soft coagulum in 90 minutes to three hours. After the addition of 0·01 c.c. of thrombin solution coagulation took place in four minutes. When, however, the thrombin solution had been previously heated it was found to have its power of producing coagulation very considerably reduced.

Thrombokinase was stated by Schmidt* to be thermostable; Morawitz,† however, asserted that thrombokinase is thermolabile; but Bordet and Delange‡ subsequently confirmed Schmidt's observation. In our experiments, as already mentioned, we have usually employed as source of thrombokinase a suspension of the stromata of the red blood cells of the rabbit, obtained by centrifugalising oxalated rabbit's blood, washing the red cells thus obtained with 0·85-per-cent. sodium chloride solution, and then laking by the addition of an equal volume of distilled water; in other experiments, however, peptone has been employed as source of thrombokinase.

The coagulant effect of red cell stromata in presence of calcium chloride is shown in the first table on p. 186.

It will be observed that heating had no marked effect upon thrombokinase, though the liquid employed as source of thrombokinase was thereby rendered turbid. The coagulum formed was in all cases firm.

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* A. Schmidt, 'Zur Blutlehre,' Leipzig, 1892; 'Weitere Beiträge zur Blutlehre,' Wiesbaden, 1895.

† Morawitz, 'Hofmeister's Beitr.,' 1903, vol. 4, p. 381.

‡ J. Bordet and L. Delange, *loc. cit.*

Fibrinogen solution.	Thrombokinase (suspension of red-cell stromata of rabbit).	0·6-per-cent. $CaCl_2$ solution.	0·85-per-cent. NaCl solution.	0·83-per-cent. solution of potassium oxalate.	Coagulation time (30° C.).
c.c.	c.c.	c.c.	c.c.	c.c.	
0·2	—	0·03	0·27	—	90 minutes.
0·2	0·01	0·03	0·26	—	21 ,,
0·2	0·03	0·03	0·24	—	7 ,,
0·2	0·10	0·03	0·17	—	6 ,,
0·2	0·10*	0·03	0·17	—	8 ,,
0·2	—	—	0·27	0·03	3 hours.
0·2	0·01	—	0·26	0·03	3 ,,
0·2	0·03	—	0·24	0·03	2¼ ,,
0·2	0·10	—	0·17	0·03	2¼ ,,
0·2	0·10*	—	0·17	0·03	2½ ,,

* Previously heated to 60° for 5 minutes.

The effect of heat upon a peptone solution is thus exhibited :—

Fibrinogen solution.	Thrombokinase (5-per-cent. solution of peptone).	0·6-per-cent. $CaCl_2$ solution.	0·85-per-cent. NaCl solution.	0·83-per-cent. solution of potassium oxalate.	Coagulation time (30° C.).
c.c.	c.c.	c.c.	c.c.	c.c.	
0·2	—	0·03	0·27	—	90 minutes.
0·2	0·01	0·03	0·26	—	12 ,,
0·2	0·03	0·03	0·24	—	15 ,,
0·2	0·10	0·03	0·17	—	20 ,,
0·2	0·30	0·03	—	—	50 ,,
0·2	0·01*	0·03	0·26	—	15 ,,
0·2	—	—	0·27	0·03	3 hours.
0·2	0·01	—	0·26	0·03	2½ ,,
0·2	0·03	—	0·24	0·03	2½ ,,
0·2	0·10	—	0·17	0·03	2½ ,,
0·2	0·30	—	—	0·03	2½ ,,
0·2	0·01*	—	0·26	0·03	2½ ,,

* Previously heated to 60° for 5 minutes.

With the addition of increasing amounts of peptone in presence of calcium chloride delay of the onset of coagulation was observed, the coagula being very soft.

The Action of Viper Venom upon Circulating Blood Plasma.

The lethal amount of the venom of *Echis carinatus* (administered by intravenous injection) was found by C. J. Martin to be for the rabbit about half a milligramme per kilogramme of body weight; if a large dose of viper venom was injected rapidly into a vein, intravascular clotting occurred; if a relatively small dose was injected very slowly, the coagulability of the blood was diminished. It may here be observed that very small doses (0·03 mgrm. or less per kilogramme of body weight) fail to produce any recognisable ill effect in rabbits.

The effect of intravenous injection of viper venom upon circulating blood plasma was studied in a series of experiments similar to the following:—

Experiment 4.—A rabbit weighing 384 grm. received into the vein of the ear an injection of 2 c.c. of 0·65-per-cent. sodium chloride solution containing 0·13 mgrm. of viper venom (0·34 mgrm. per kilogramme of body weight). Five minutes after the completion of injection, which occupied one minute, the animal, which had not up to this time been obviously affected, showed signs of feebleness, and then became convulsed, death occurring about three-quarters of a minute later. At the *post-mortem* examination, which was made without delay, the blood in the superior vena cava, the right auricle and ventricle of the heart and the pulmonary artery was found to be clotted, the clot extending into the vein injected; on the left side of the heart, in the aorta, portal vein, and lower part of the vena cava, liquid blood was found; the lungs collapsed normally on opening the chest.

On microscopical examination of the lungs after death by viper venom it was found that in all cases, no matter whether intravascular coagulation resulted (four experiments) or the blood coagulated slowly (three experiments) or remained permanently liquid (three experiments), fibrin masses, filaments, and fibrils could be readily recognised. The appearance presented by the collections of fibrin was exactly the same as that presented when relatively large doses of thrombin solution had been injected, so that it was not possible from an examination of the lungs to state whether the coagulant injected had been the latter or the former. The diminished coagulability or "negative phase" exhibited after the intravenous injection of viper venom is therefore dependent upon the removal of fibrinogen under the action of the coagulant. As already mentioned, Martin* observed that the slow injection in the dog of small quantities (*i.e.* below 0·1 mgrm. per kilogramme of body weight) of viper (*Pseudechis porphyriacus*) venom resulted in the production of liquid blood, and it is easy to understand how this procedure, by allowing time for the contraction of the delicate fibrin network, which is at first formed, into dense masses of relatively small size, would cause far less blocking of vessels and resulting interference with the circulation than would the rapid injection of the same or smaller amounts of venom. Nevertheless, in my experiments upon rabbits, liquid blood has followed the injection (in the course of about one minute) of relatively large quantities of venom (3·4–0·6 mgrm. per kilogramme of body weight). Liquid blood, moreover, does not exclude the presence of visible clot in the larger vessels. Thus in the experiment referred to at the close of the next section, in which 0·73 mgrm. of venom per kilogramme of body weight was injected into a rabbit, it was found that the heart was continuing to beat feebly and slowly after respiration had stopped and all movements of the voluntary muscles had ceased. On opening the right ventricle 5 c.c. of blood was obtained, which remained permanently liquid. The vein of the ear, external jugular, superior and inferior caval veins, portal and renal veins were all distended with clot; nevertheless, obstruction to the circulation was not complete, and blood was delivered from the incised right ventricle at each beat. In the blood-vessels of the lungs fibrin filaments and masses were abundant.

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* C. J. Martin, 1893, *loc. cit.*, p. 382.

That the appearance of liquid blood after injection of viper venom is dependent upon removal of fibrinogen is shown not only by the appearance of fibrin in blood-vessels, particularly of the lungs, but also by the result of examination of the fibrinogen content of the liquid blood. Thus it was found that when one part of the thrombin solution employed in the preceding section was mixed with two parts of the liquid blood obtained in the experiment just described, coagulation did not occur, though the thrombin solution was capable of coagulating two parts of fibrinogen solution in about three-quarters of a minute. The liquid blood was, however, found to contain thrombin, for when added to two parts of fibrinogen solution coagulation occurred at the end of one and a half minutes; the same result was obtained if one-fifteenth part of 0·83-per-cent. potassium oxalate solution had also been previously added. The thrombin in question cannot have been produced by the action of venom upon fibrinogen, for serum expressed from a coagulum so obtained, as, for example, in the second experiment of the series given on p. 189, exhibits the usual character of serum from a clot produced by the action of thrombin upon fibrinogen solution under similar conditions of experiment, that is to say, it possesses scarcely any recognisable coagulant action on fibrinogen. It follows, therefore, that the thrombin contained in the liquid blood consists of venom. In the experiment in question the amount of venom injected into the blood represented 1 part in 70,000 parts of blood. In the next section it is shown that 1 part in 15,000,000 is capable of coagulating a solution containing fibrinogen in approximately two-fifths of the concentration present in blood plasma; it is therefore to be expected that part of the venom injected would remain unchanged in the liquid blood, thereby conferring upon it a coagulant action when added to fibrinogen solution.

Even when, after the injection of a lethal dose of viper venom, clotting occurs in the heart and great blood-vessels, the ease with which fibrin masses can be recognised in the smaller pulmonary vessels renders it possible readily to distinguish between the effect of injection of thrombin or viper venom and that of a relatively large amount of thrombokinase contained in red-cell stromata or peptone. It is obvious from the effect of injection of viper venom into the blood stream that the coagulant in the venom is a thrombin and not a thrombokinase.

The Effect of Heat upon Viper Venom.

The blood coagulant of the venom of the Indian viper, *Echis carinatus*, is completely destroyed by heating to 75° C. for 10 to 15 minutes.* The following experiments exhibit the coagulant activity of this venom before and after heating:—

Fibrinogen solution.	Solution of viper venom 1 in 300,000 of 0·85-per-cent. NaCl.	0·6-per-cent. $CaCl_2$ solution.	0·85-per-cent. NaCl solution.	0·83-per-cent. solution of potassium oxalate.	Coagulation time (30° C.).
c.c.	c.c.	c.c.	c.c.	c.c.	
0·2	—	0·03	0·27	—	90 minutes.
0·2	0·01	0·03	0·26	—	10 ,,
0·2	0·03	0·03	0·24	—	5 ,,
0·2	0·10	0·03	0·17	—	4 ,,
0·2	0·30	0·03	—	—	3 ,,
0·2	0·30*	0·05	—	—	53–60 ,,
0·2	—	—	0·27	0·03	3 hours.
0·2	0·01	—	0·26	0·03	16 minutes.
0·2	0·03	—	0·24	0·03	10 ,,
0·2	0·10	—	0·17	0·03	7 ,,
0·2	0·30	—	—	0·03	4 ,,
0·2	0·30*	—	—	0·03	60 ,,

* Heated to 75° for 10 minutes.

It will be seen that viper venom, even when present to the extent of only 1 part in 15,000,000, is capable of coagulating in ten minutes at 30° C. a liquid containing approximately the same concentration of fibrinogen as normal rabbit's plasma. When heated, however, its coagulant action rapidly disappears.

It is obvious from the effect of heating that the coagulant of viper venom cannot be regarded as thrombokinase as Mellanby† has suggested. Its behaviour in respect of heat shows it to be a thrombin.

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* C. J. Martin, 1905, *loc. cit.*

† J. Mellanby, *loc. cit.*, p. 467.

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The intravenous injection of heated venom, even in the amount of 1·1 mgrm. per kilogramme of body weight, was found to be without effect upon rabbits; examination of sections of the lungs and also of the liver, spleen, kidney, and heart muscle of animals killed half an hour after injection failed to reveal any separation of fibrin in the blood-vessels. A control experiment, in which 0·73 mgrm. per kilogramme of body weight was injected in the course of one minute, caused death at the end of 30 seconds. The behaviour of heated venom, when injected into the blood stream, is thus seen to be consistent with the conclusion that the coagulant is a thrombin.

Summary.

The different mode of action exerted by thrombin and thrombokinase upon circulating blood plasma is described, and it is shown that the coagulant of viper (*Echis carinatus*) venom, as exhibited by its effect in causing intravascular separation of fibrin when injected into the blood stream, and also indicated by its behaviour when heated, is a thrombin and not a thrombokinase.

Negative After-Images and Successive Contrast with Pure Spectral Colours.

By A. W. Porter, B.Sc., F.R.S., Fellow of University of London University College, and F. W. Edridge-Green, M.D., F.R.C.S.

(Received March 31,—Read November 13, 1913.)

In a recent paper[*] Prof. Burch has criticised our results on "Negative After-Images and Successive Contrast with Pure Spectral Colours."[†] Prof. Burch suggests that the change in blue and violet obtained after fatigue with red light may be explained on the Young theory, if the stray light, which we stated was present, be taken into consideration. He states that the reason, on this theory, why the violet appeared bluer and darker after fatigue to red was due to the elimination of the red component in the stray light.

In consequence of this criticism we have since repeated our experiments, taking the most minute precautions to exclude stray light by covering the whole apparatus and head of the observer with black velvet. When these most minute precautions were taken to prevent the admixture of red or other light the results were exactly the same as before. The experiments were conducted as follows: A region of pure violet, λ 4368–λ 4572, was isolated in the Edridge-Green spectrometer, a deep blue-green glass quite opaque to red being placed in front of the slit, so that no red light could enter the instrument. A region of pure red, λ 6360–λ 6570, was isolated in another spectrometer, deep ruby glass being placed in front of the slit so that nothing but red light could enter the instrument. The eye was then fatigued as before, one eye being vertically above the other, for 20 seconds, and the after-image projected upon a narrow vertical band in the violet region after turning the eyes round into the normal position, so that the two images crossed at right angles. The result was exactly the same as stated previously by us, the region of violet crossed by the after-image appeared bluer and darker.

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[*] 'Roy. Soc. Proc.,' 1913, B, vol. 86, p. 117.

[†] 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 434.

It should be here noted that when the red band was intently regarded for 10 seconds and the eye then slightly moved (to another part of the same telescopic field) a bright blue-green after-image was visible, although the only light then being received by the eye was red light.

The experiment with yellow light on a screen was repeated in the spectrometer with exactly the same result. Pure yellow light, λ 5820–λ 5870, was isolated in one spectrometer, and red light, λ 6360–λ 6570, used to fatigue the eye. The results were as before, the yellow appeared unchanged, or, when the exciting light was comparatively intense, slightly greener and darker in the region of the after-image, whilst a deep blue-green after-image extended on either side.

These experiments show that the stray light, mentioned by us in our former paper, was of negligible amount; for we have now obtained precisely the same results when stray light was most rigorously excluded. Stray light, of amount comparable with that in our previous experiments, is present in all spectrometric investigations unless precautions such as those described above are taken.

The Ratio between Spindle Lengths in the Spermatocyte Metaphases of Helix pomatia.

By C. F. U. Meek, M.Sc., F.L.S., F.Z.S.

(Communicated by Sir W. T. Thiselton Dyer, K.C.M.G., C.I.E., F.R.S. Received July 15,—Read December 4, 1913.)

[Plate 12.]

Introduction.

I have recently shown that in *Forficula auricularia* the length of the mitotic spindle, *i.e.* the distance between the centrosomes, seems to be a constant at the conclusion of each spermatocyte metaphase. The ratio between the lengths found at this stage is almost identical with the ratio between the radii of two spheres of which the volume of one is equal to twice that of the other; and, since the volume of the primary spermatocyte cell in the metaphase is presumably equal to twice that of the secondary spermatocyte, connection is suggested between the length of the spindle and the volume of the cell.

I now propose to measure spindle lengths in the spermatocyte metaphases of *Helix pomatia.* As in the case of Forficula, the chromosomes are spheres or very short rods, and all seem to divide on the spindle at the same time; the conclusion of each metaphase is therefore easily recognised. If the lengths are found to be constants, and if the ratio between them is approximately 1·26 : 1, the connection between spindle length and cell volume is again suggested: if, on the other hand, lengths are not constants, or if the ratio between them is not approximately that mentioned above, the suggested connection is at once disproved.

Material and Methods.

The material, which consisted of the hermaphrodite gland, was obtained at the end of May, and was preserved in Flemming's strong chromo-aceto-osmic acid fluid and the platino-aceto-osmic acid fluid of Hermann. The material remained in the fixative for 12 hours, and, after being washed thoroughly in running water and passed through successive strengths of alcohol, was embedded in paraffin. Sections were cut 8 μ thick with an ordinary Cambridge rocking microtome.

The stains used were Heidenhain's iron hæmatoxylin and iron brazilin,* and both have given excellent results. The latter, which affects the cytoplasm as well as the chromatin, enables spindle fibres to be seen very distinctly; and all drawings on the plate have accordingly been made from sections thus stained. In the case of the iron hæmatoxylin, the slides were placed for four hours in the mordant, which was an aqueous solution of ferric alum, and were then stained for 12 hours; in the case of the iron brazilin, the slides remained for two hours in a solution of ferric alum in 70-per-cent. alcohol, and were then placed in the stain for 15 hours.

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* Hickson, S. J., 'Quart. Journ. Micro. Sci.,' 1901, vol. 44.

The preparations were studied with a Zeiss apochromatic oil-immersion objective of 2 mm. focus and N.A. 1·30, and compensating oculars Nos. 4, 6, 12, and 18. The light was obtained from an inverted incandescent gas burner, and was passed through a Gifford screen and the holoscopic oil-immersion substage condenser of Messrs. Watson and Sons, of London. All drawings were made with a large Abbé camera lucida at one magnification, which was estimated with a stage micrometer graduated to read one-hundredth part of a millimetre. Possible distortion was prevented by levelling the microscope platform and drawing table; and, in order to minimize error due to foreshortening, measurements have been made only of spindles of which the major axes lay at right angles to the microscopic line of vision, *i.e.* spindles of which the centrosomes could be brought into focus simultaneously. I have tried to eliminate inaccuracy of draughtsmanship by drawing the centrosomes of each spindle many times and upon several occasions; moreover, the lengths found by me have been checked by independent measurements made by my assistant, Mr. Russell Goddard.

The Length of the Mitotic Spindle at the Conclusion of the Primary Spermatocyte Metaphase.

Fig. 1 of the plate represents a polar view of the primary spermatocyte complex. I have not attempted to count the chromosomes on the various spindles; but recent investigations seem to show that the number is 48 in the spermatogonial and 24 in the spermatocyte cells. The chromosomes are short thick rods, and do not differ from one another greatly in size.

Figs. 2 to 12 inclusive are drawings of lateral views of the spindle at the conclusion of this metaphase; in each figure constriction of the chromosomes is seen to have been completed, and the daughter rods, apposed to one another in the equatorial plane, are ready to move apart. These drawings have been made at a magnification of 650 diameters from sections in the hermaphrodite glands of several individuals, and the length of the spindle is the same in all. This length has been found in every primary spermatocyte cell studied at this stage, and, at the known magnification, represents 15·3 μ.

In the circumstances we have reason for believing that the length of the spindle is a constant at the conclusion of the primary spermatocyte metaphase.

The Length of the Mitotic Spindle at the Conclusion of the Secondary Spermatocyte Metaphase.

Fig. 13 represents a polar view of the equatorial plate in this metaphase. The chromosomes are noticeably smaller than those of the preceding cell generation.

Figs. 14 to 23 inclusive are drawings of lateral views of the spindle at the conclusion of the metaphase, *i.e.* at the moment when constriction of the chromosomes is complete. As in the case of figs. 2–12, these drawings have been made at a magnification of 650 diameters from sections in the hermaphrodite glands of several specimens. The length of the spindle, estimated from the magnification, is invariably 12·1 μ; and, since the centrosomes have been found to be equidistant in all secondary spermatocyte cells studied at this stage, we seem again to be dealing with a constant.

The Ratio between the Lengths of the Mitotic Spindle at the Conclusion of the Primary and Secondary Spermatocyte Metaphases.

I have already remarked that in *Forficula auricularia* the ratio between the lengths of the mitotic spindle at the conclusion of the two spermatocyte metaphases is almost identical with the ratio between the radii of two spheres of which the volume of one is equal to twice that of the other. The former ratio is 1·28 : 1·00, and the latter ratio is 1·26 : 1·00.

Now the lengths of the spindle found for the conclusion of these metaphases in *Helix pomatia* are 15·3 and 12·1 μ respectively, and the ratio between them is 1·26 : 1·00. No period of growth separates the primary and secondary spermatocyte mitoses in this organism; the connection between spindle length and cell volume is therefore again suggested.

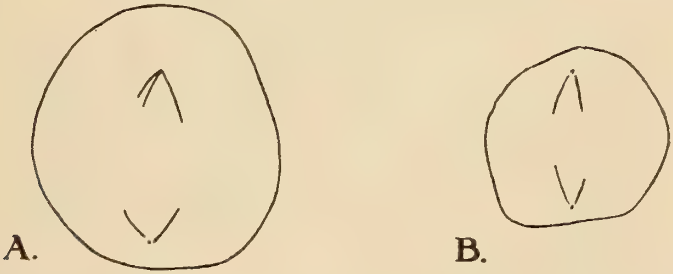
The accuracy of my measurements seems to be confirmed by the work of Demoll. In a paper published last year upon the spermatogenesis of *Helix pomatia*, he gives two drawings respectively representing the lengths of the mitotic spindle in the primary and secondary spermatocyte metaphases. We are not told at what stage of the metaphase these drawings were made, nor is the magnification mentioned; but the lengths shown are 29·7 and 24·0 mm., and the ratio between them is 1·24 : 1·00. Demoll, however, appears to have seen no possible significance in these relative lengths; for he dismissed the matter by saying that the length of the spindle decreases only slightly when the cell volume is halved.

Measurements of Spindle Lengths made by an Independent Investigator.

During this research I wrote upon the subject to Dr. von Winiwarter, who very kindly offered to measure spindle lengths in the spermatocyte metaphases of man.

I have since received a letter, in which he says: "J'ai effectué une série de mensurations sur les fuseaux des spermatocytes I et II chez l'homme, et constaté qu'il y a une légère différence de longueur entre les deux. Cette différence est trop faible pour être reconnue autrement que par des mensurations. Je vous envoie en même temps quelques uns des croquis qui m'ont servi à calculer les rapports. Ils sont faits à un grossissement de 2400 diamètres avec le système d'ocul. et d'object. employé pour tous les dessins de mon travail sur la spermatogenèse humaine ('Arch. de Biol.'). J'ai simplement indiqué les corpuscles centraux, le début du fuseau et les contours du corps cellulaire. Je n'ai pas dessiné les chromosomes; ceux-ci sont exactement au moment où ils sont rangés régulièrement à l'équateur et vont se diviser. Les fuseaux sont bien parallèles à la table du microscope et dans une seule coupe. Vous constaterez vous même que l'analogie avec forficula est tellement complète que le rapport entre les fuseaux I et II est aussi 1·26 : 1·00."

The camera lucida drawings enclosed in this letter represent five primary and five secondary spermatocyte metaphases. The length of the spindle is 24 mm. in each of the former, and 19 mm. in the latter; and, since the magnification is 2400 diameters, the lengths in the cell must be 10·0 and 7·9 μ respectively. Examples of these drawings are given below at a slightly reduced magnification. In the circumstances the ratio that I have observed in *Forficula auricularia* and *Helix pomatia* is shown to exist in material belonging to a third phylum; and I take this opportunity of again thanking Dr. von Winiwarter for his kindness in making the measurements and allowing me to publish the results.



Camera lucida drawings of spindles in the spermatocyte metaphases of Man.
A. Primary spermatocyte. B. Secondary spermatocyte. × 2270.

Conclusion.

Whether the connection suggested between spindle length and cell volume in the metaphase is likely to be established or not is impossible for us to say: the proposition that I have put forward is at present entirely speculative. But the results of research have shown that at the stage in question the ratio between spindle lengths is approximately the same in the spermatocytes of *Helix pomatia*, *Forficula auricularia*, and man—organisms representing three phyla of the animal kingdom.

Moreover, consideration of the lengths found in these organisms proves that the length of the spindle in the metaphase cannot be correlated with the volume of the chromatin. This is important; for in an earlier paper I have produced evidence to show that increasing somatic complexity is accompanied by increase of chromatin volume in the cell.

The failure of current theories of mitosis is largely due to the absence of data from which to draw conclusions; and, since either proof or disproof of my proposition must constitute a new generalisation, I intend to carry out further and similar cytometrical investigations, of which the results will appear in subsequent papers.

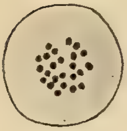
Summary.

1. The length of the mitotic spindle, *i.e.* the distance between the centrosomes, is 15·3 μ at the conclusion of each primary spermatocyte metaphase of *Helix pomatia*.

2. The length of the mitotic spindle is 12·1 μ at the conclusion of each secondary spermatocyte metaphase of *Helix pomatia*.

3. The ratio between the lengths of the mitotic spindle at the conclusion of the primary and secondary spermatocyte metaphases is approximately the same in *Helix pomatia*, *Forficula auricularia*, and man; and, since these ratios are either identical or almost identical with the ratio between the radii of two spheres of which the relative volumes are the same as those of the cells in question, connection may exist between spindle length and cell volume at this stage.

4. A comparison of mitotic figures in *Helix pomatia*, *Forficula auricularia*, and man proves that the length of the spindle in spermatocyte metaphases cannot be correlated with the volume of chromatin in the cell.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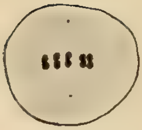
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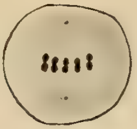
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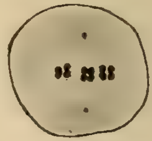
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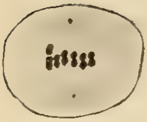
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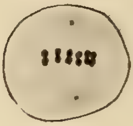
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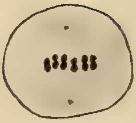
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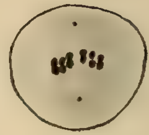
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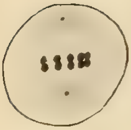
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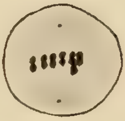
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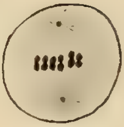
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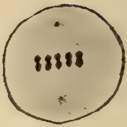
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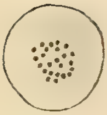
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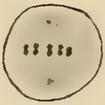
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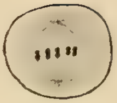
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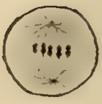
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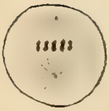
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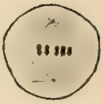
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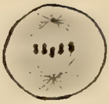
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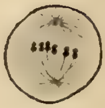
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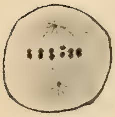
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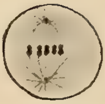
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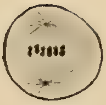
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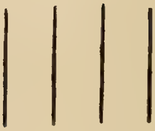
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EXPLANATION OF THE PLATE.

Fig. 1.—Polar view of primary spermatocyte complex.

Figs. 2–12.—Lateral views of spindle at conclusion of the primary spermatocyte metaphase, showing completed constriction of chromosomes. In each figure the length of the spindle, estimated from the magnification, is 15·3 μ.

Fig. 13.—Polar view of secondary spermatocyte complex.

Figs. 14–23.—Lateral views of spindle at conclusion of the secondary spermatocyte metaphase, showing completed constriction of chromosomes. In each figure the length of the spindle, estimated from the magnification, is 12·1 μ.

Fig. 24.—Divisions of stage micrometer, 10 μ apart, showing magnification of figs. 1–23 inclusive.

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* A list of publications dealing with the spermatogenesis of Helix is given in the bibliography of this paper.

Neuro-Muscular Structures in the Heart.

By A. F. Stanley Kent, M.A. Oxon., Professor of Physiology, University of Bristol.

(Communicated by Prof. C. S. Sherrington, F.R.S. Received July 25,—Read November 20, 1913.)

(From the Physiological Laboratory of the University of Bristol.)

The fundamental fact of the existence of a muscular connection between auricle and ventricle in the mammalian heart was established in 1892 (5, 6). The details of the particular connection first studied were worked out during the years following (1, 3, 7, 8, 13, 15), and attention was directed so completely to the auriculo-ventricular bundle itself that additional ties between auricle and ventricle at other points remained relatively neglected. Partly in result of this, though partly in result of experiments which have been perhaps imperfectly understood, an impression has gained ground (2, 14) that apart from the originally described auriculo-ventricular bundle there exists no other conducting path capable of transferring the state of activity from auricular muscle to ventricular, or *vice versâ*. This impression has, indeed, been put forward as an actually ascertained fact (10).

For some years, however, a mass of facts has been accumulating difficult to explain on the supposition that the conduction between auricle and ventricle consists of one single path alone. The facts can, on the other hand, be explained satisfactorily if there be granted the existence of an auriculo-ventricular connection which is multiple.

These facts have become known partly as the result of clinical experiences and partly as the result of direct experiment, and are so definite that it is necessary for any satisfactory theory of the cardiac mechanism to take account of them.

The clinical experiences referred to fall into two categories:—

A. Cases in which the auriculo-ventricular sequence was found to be normal, though the bundle was destroyed (4, 11); and

B. Cases in which the auriculo-ventricular sequence was abolished, though the bundle was intact (4, 9, 12).

There are in the literature several cases illustrating each of these conditions, and the conclusion is becoming more and more firmly established, that the normal auriculo-ventricular sequence may exist with a destroyed bundle, and that the sequence may be disturbed, or abolished, the bundle remaining unaffected.

In other words, it appears that the auriculo-ventricular bundle is not the only path by which the functional connection between the auricle and ventricle may be established, and that "the co-operation between the auricle and ventricle is not necessarily dissolved because the auriculo-ventricular bundle has been put out of action" (4).

The experimental evidence to which reference has been made is at present unpublished, and was first brought to my notice by Prof. Leonard Hill, to whom I am indebted for permission to refer to it. I have recently repeated the experiments, and can have no doubt as to their real significance.

The evidence is of the following character:—

If in the beating heart of a mammal the anatomical connections between the left auricle and left ventricle are severed, and the section is carried through the septum also, thus leaving only the right ventricular wall attached to the auricle, even under these circumstances co-ordinated beats pass over the auriculo-ventricular junction, the ventricular contraction following the auricular in its proper sequence.

With such clinical and experimental evidence before us it is idle to assert that no conducting path exists other than the well-known and well-defined auriculo-ventricular bundle, and the question is no longer "Does a connection exist?" but "What is the nature of the connection?"

It may perhaps be recollected that as long ago as 1892 (5, 6) I described the existence of a connection in this situation, viz., between the outer wall of auricle and ventricle. The importance of the recently-described septal connections overshadowed this other observation, however, and its significance was not appreciated. It was only when the fact that the auriculo-ventricular bundle could be destroyed without abolishing the co-ordinated action between the chambers that the importance of additional conducting paths was brought into prominence.

During the past few years my work on the human heart has shown that there exists a mechanism which may perhaps help to elucidate the manner in which these hitherto imperfectly explained transferences of activity are brought about, and although the details have not all been worked out, it may perhaps be of use to place the facts on record.

It is well known that in a series of sections made through the auriculo-ventricular junction an outstanding feature is the large number of nervous structures present. It is no uncommon thing to find from 20 to 30 nerve trunks cut across, some $50\,\mu$ to $100\,\mu$ in diameter, most of them lying in the fat and connective tissue of the groove, whilst in addition to these there are trunks of large size lying amongst the muscular tissue, and apparently derived directly from those in the groove.

In close association with these nerve fibres there exist nerve cells, occasionally single, often in groups of two or three, sometimes in large numbers. Many of these cells are of great size, and in other particulars remarkable in appearance. They are very markedly irregular in their distribution, and may be scanty even where nerve fibres are abundant, whilst in other situations they may be numerous.

The exact function of these nerve cells is open to conjecture, and it is therefore of interest to find them associated with other structures, the connections of which may throw light upon their mode of action.

The structures referred to are found lying in the connective tissue between the auricular and ventricular muscle, and are of a type hitherto undescribed in the heart. They consist of an elongated body, the first indications of which in any series of sections is the appearance of two or three nerve fibres lying amongst the fibrous tissue. In sections passing directly from auricle to ventricle and taken vertically to the surface, these fibres are as a rule cut transversely. If the series be followed the number of fibres in the group is seen to increase, until ultimately a large number are present.

At this point some resemblance to an ordinary nerve trunk is presented, and the diameter of the structure may be about 170 μ.

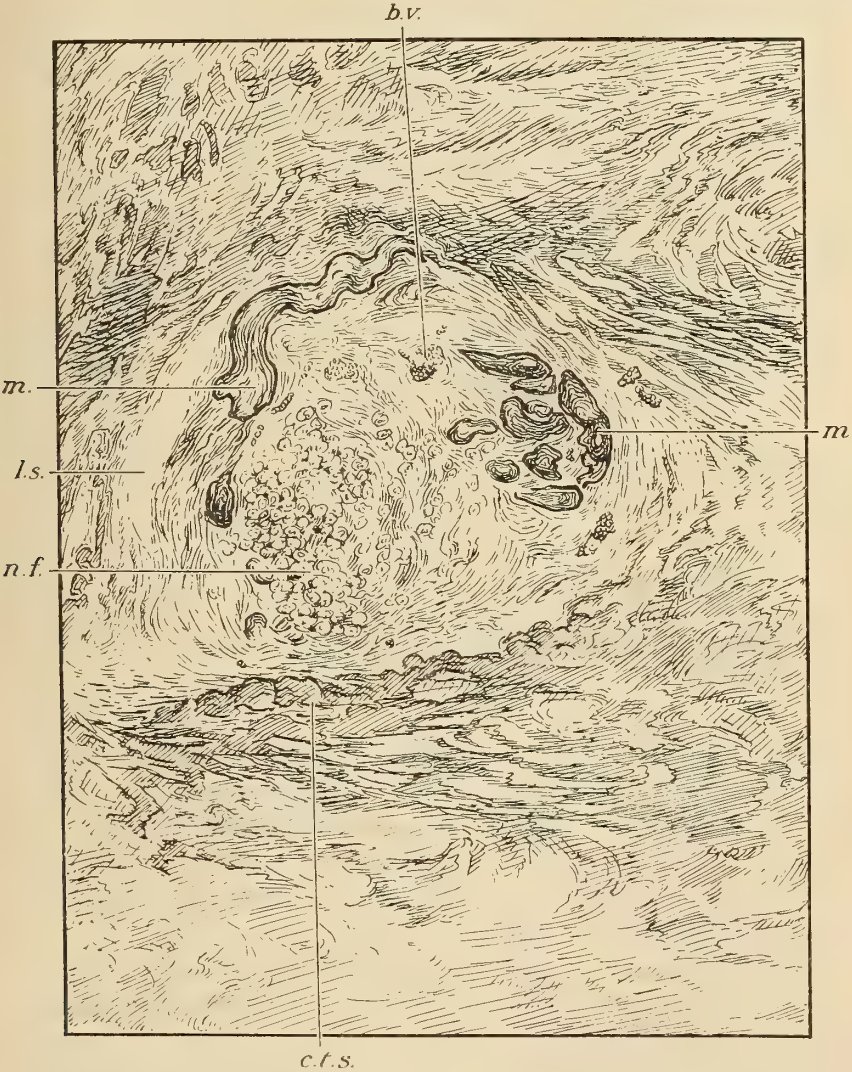
The constituent fibres vary a good deal in size, ranging from about 3 μ to about 12 μ in diameter. A number of measurements gave the average diameter of the most numerous fibres as about 7 μ. Connective tissue of the fibrous variety is present in the bundle, but is principally developed at the periphery, where in the greater portion of the length of the bundle, a definite sheath is present, of considerable thickness, and composed of many layers with large lymphatic spaces lying between them.

If the bundle, for the structure has now assumed the form of a bundle, be followed further, a new constituent will be noticed to have made its appearance. This new constituent is muscle, and it generally appears as a small mass of tissue which stains more deeply than the rest of the bundle, and is readily distinguished from the other constituents present.

The muscle, after its appearance in the bundle, is generally to be found as a fibre running longitudinally, or winding amongst the other tissues, and showing a sharp differentiation into the darkly staining sarcostyles, and the lighter sarcoplasm. The latter is generally arranged at the centre of the fibre, whilst the sarcostyles occupy the periphery.

One muscle fibre having appeared, others are soon noticeable, and the number increases until a considerable portion of the bundle may be occupied by muscular tissue.

At the same time that the muscle becomes prominent in a bundle it may be noticed that a very large increase in the blood supply has taken place, and that, instead of the occasional small vessel observed at the commencement, a rich supply of blood is brought to the bundle by vessels of considerable size, and distributed through its substance. It may be stated generally that the blood supply is found to be most abundant in those



Transverse section of structure lying in the connective tissue between the auricle and ventricle of human heart. × about 300. *c.t.s.*, connective tissue sheath; *l.s.*, lymph space; *b.v.*, blood vessel; *n.f.*, nerve fibres; *m.*, muscle. The general relations of parts only are shown, minute detail being for the most part omitted.

situations where the muscular tissue forms a constituent portion of the bundle.

With regard to the character of the muscle fibres which are found in the bundle, these appear to be of two kinds. Where the muscle is well represented some fibres will usually be found to resemble the tissue present in the neighbouring chamber of the heart. Others, however, are of a different type, and appear as large pale fibres, with but few sarcostyles, those that are present being grouped around the periphery of the fibre.

If the series of sections be followed further it will be found that the bundle, which at first may be at some distance from the muscle of the heart chamber, gradually approaches this latter, whilst at the same time some of the large pale fibres alluded to as being present in the bundle will be observed to be making their appearance also in the auricular or ventricular tissue, and, after a time, a definite exchange of muscle fibres on a large scale will be observed to take place between the auricle, or ventricle, and the bundle. Fibres which are apparently normal auricular or ventricular tissue approach the connective tissue, pass through as a definite mass of tissue, penetrate the connective tissue sheath of the bundle, and come to lie in its interior. In many cases the amount of muscle entering the bundle in this way is considerable, and much of the tissue is indistinguishable from ordinary cardiac tissue. Some, however, is of the character already described, consisting of fibres with clear centres and sarcostyles scattered around the periphery, and, though smaller, presenting some of the characters of Purkinje fibres.

It follows from this description that the amount of muscle present in the bundle varies considerably from place to place, and, moreover, that the character of the muscular tissue varies also, being sometimes similar to auricular or ventricular tissue, and having similar staining properties, and sometimes pale fibres containing much faintly staining sarcoplasm with comparatively few darkly staining sarcostyles.

The nerve fibres which form so important a part of the structures described are of various sizes, from $3\,\mu$ to $12\,\mu$. They run, as a rule, a longitudinal or somewhat winding course in the bundle, and may be demonstrated to be connected at various points with the nervous structures lying in the fat at the auriculo-ventricular junction. They may also be traced leaving the bundle at various points, and passing away through the connective tissue towards the neighbouring tissues.

From the description which has been given it is apparent that the structure which has been described presents itself as an elongated body of different diameters at different parts of its course, and therefore of a conical or fusiform shape, into the composition of which both nerve fibres and muscle fibres of two distinct varieties enter, surrounded by a distinct connective tissue sheath in which large lymphatic spaces exist, and being abundantly supplied with blood. Further, that these structures can be shown to be connected with the muscular tissue of the auricle or of the ventricle on the one hand, and with the nervous structures lying in the auriculo-ventricular groove on the other.

It will be at once apparent that the structures described have many points in common with the neuro-muscular spindles found in skeletal muscle. The association of nerve and muscle fibres in a definite structure, the modification of the muscle fibres, the general shape, even the connective tissue sheath with its lymphatic spaces, all recall the structure of neuro-muscular spindles.

And it may be that, just as the structure of the two organs is similar, so also are their functions. In the neuro-muscular spindles of skeletal muscle we see organs destined for the reception of impulses from the muscle fibres—impulses which pass to a centre consisting of nerve cells and throw it into activity, *i.e.* the organ is a receptive one and functions as a part of a reflex arc.

In the neuro-muscular structures described we see organs which from their structure and connections may well function as receptive organs, which may well be roused to activity by the muscle, and transmit impulses, it may be, to the local centre, *i.e.* to the nerve cells in the auriculo-ventricular groove.

And, further, if this is so, it is difficult to avoid the suggestion that we see here, as yet imperfectly described and obscure in some of its workings, a local mechanism whose function it is to place in communication the various chambers of the heart, and to correlate their activities—a mechanism consisting of receptive organ, afferent path, centre, efferent path, and distributing organ, and constituting a local reflex arc, which may perhaps exhibit only an occasional activity, the co-ordination of the cardiac rhythm being, as a rule, provided for by the muscular connections of the auriculo-ventricular bundle, but which may be capable of controlling that co-ordination when the bundle is no longer perfect.

This research has been assisted by a grant from the Research Fund of the University Colston Society.

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The Alleged Excretion of Creatine in Carbohydrate Starvation.

By George Graham, Beit Memorial Fellow, and E. P. Poulton, Radcliffe Travelling Fellow.

(Communicated by Dr. F. G. Hopkins, F.R.S. Received August 5,—
Read November 20, 1913.)

(From the Pathological Department, St. Bartholomew's Hospital, and the Physiological Department, Guy's Hospital.)

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Introduction.

It was stated by Cathcart (4) and Benedict and Myers (2) independently, in 1907, that creatine was excreted in the urine during inanition. Cathcart (5) has further stated that the output of creatine, caused by fasting for 36 hours, is diminished as soon as a diet consisting of carbohydrates is taken, whereas it is increased by a fat diet.

Rose and Mendel (19) confirm these results, laying great stress on the fact that carbohydrates play a very important *rôle* in preventing the excretion of creatine in the urine.

In the course of a 10 days' experiment on one of us (G. G., 10), where the diet was restricted to protein and fat and was of insufficient calorie value, we found that no creatine was excreted in the urine. The explanation of this discrepancy was not fully investigated at that time, but recent work by Greenwald (12) has suggested a possible explanation.

Folin's (8) method for the estimation of creatinine in urine depends on the orange colour produced by the addition of picric acid and soda (Jaffé, 15). This colour has been shown to be due to a reducing action of the creatinine on the picric acid (Chapman, 6).

Among the reducing substances which also give a similar colour are acetone, aceto-acetic acid, and β-oxybutyric acid, all of which may be present in urine under different conditions.

Van Hoogenhuyze and Verploegh (13) and Krause (16) stated that urine to which acetone had been added produced, with picric acid and soda, a slightly darker colour than was obtained with the urine alone, but that the colour soon faded and caused no error in the determination of creatinine. Krause (16), Wolf and Osterberg (22), and Rose (20) found that the addition of the ethyl ester of aceto-acetic acid to urine did not produce any error in the estimation of creatinine, unless large amounts (*i.e.* over 1 per cent.) were added. They seem to have assumed that the action of aceto-acetic acid would be the same as that of the ester.

Recently, however, Greenwald (12), working on diabetic urines, has shown that if aceto-acetic acid is added to urine directly a considerable error is introduced into the estimation of creatinine.

This observation may possibly explain why we did not find any creatine in our experiment on the fat diet. Folin (8) originally stated that acetone and aceto-acetic acid gave the orange colour with picric acid and soda, but remarked that they could easily be removed from the urine. In our experiment we removed the aceto-acetic acid as far as possible from the urine before making the estimation, in order to get rid of any disturbing effect that the acetone bodies might have on the creatinine figures.

In the experiments described in this paper we have studied this question in greater detail and also the means of overcoming the difficulty.

I. *The Effect of Aceto-acetic Acid on the Estimation of Creatinine.*

The different intensities of colour produced by β-oxybutyric acid, acetone, aceto-acetic ester and aceto-acetic acid when treated with picric acid and soda were first investigated.

The importance of aceto-acetic acid is emphasised by the experiments of Arnold (1), Emden (7), and Hurtley (14). These investigators have, independently, pointed out that in cases of acidosis the fresh urine contains only small amounts of acetone, while aceto-acetic acid may be present in large amounts.

Throughout our experiments the estimation of the creatinine was performed in the usual way; 15 c.c. of a saturated solution of picric acid and 5 c.c. of 10-per-cent. caustic soda were added to 10 c.c. of urine, the mixture was allowed to stand for seven instead of five minutes, and then diluted to 500 c.c. with water. Folin (8) stated that the maximum intensity of colour occurs in five to nine minutes after mixing. We have always waited seven minutes because we found that five minutes was not always sufficient if the urine was slightly diluted, as occurs in the estimation of the creatinine + creatine by this method. The matching was done with a Duboscq colorimeter against an N/2 potassium bichromate solution. All the matching was done by E. P. P. while the scale was read by G. G., six to eight readings were made and the mean was taken. The total nitrogen was estimated by Kjeldahl's method. The aceto-acetic acid + acetone was estimated by the Messinger-Huppert method.

The substances tested were prepared in the following manner, and we wish to thank Dr. Hurtley for very kindly supplying us with them. The β-oxybutyric acid was extracted from urine and the strength of the solution was accurately known; the solution was nearly colourless. The acetone was chemically pure. The ethyl ester of aceto-acetic acid was obtained by distilling the pure commercial ester under reduced pressure, and the product boiled constantly at the correct boiling point for the pure substance.

The aceto-acetic acid was obtained from the ester, which was hydrolysed by adding the theoretical amount of normal caustic soda, and allowing it to stand at the room temperature for 36 hours, when the hydrolysis was complete. It will be seen that the solution used consisted of the sodium salt of aceto-acetic acid and an equivalent amount of ethyl alcohol. The mixture was diluted and the amount of acetone present was determined by Folin's method (9). The amount of aceto-acetic acid corresponding to the acetone present was deducted from the theoretical amount of aceto-acetic acid in order to get the correct value for the aceto-acetic acid. The aceto-acetic acid was kept in an ice chest in order to prevent its decomposition, and was tested from time to time by means of the Folin and Messinger-Huppert methods.

β-oxybutyric acid, acetone, the ethyl ester and the sodium salt of aceto-acetic acid all give an orange colour when treated alone with picric acid and soda, but on dilution the solution is much paler than the usual colour obtained with urine under these conditions. These substances were then added to urine in varying concentrations, and the colour produced by the addition of picric acid and soda was compared with the colour produced by the urine alone with the picric acid and soda, without the addition of these substances. The addition of β-oxybutyric acid produces practically no alteration in the colour obtained by adding picric acid and soda to urine.

Thus when added to the urine (Table I) in amounts corresponding to 0·036 grm. per 100 c.c. and 1 grm. per 100 c.c. it caused no error at all in the creatinine determination. When present in amounts corresponding to a 2·16-per-cent. solution it made the colour slightly lighter, causing a difference of 0·27 mm. scale reading, which is almost within the limit of accuracy of the method. The amounts of the β-oxybutyric acid added are quite comparable with those found in the urine in diabetes, and as the error caused even by large amounts is so small its effect may be safely neglected.

Table I shows the Effect of Increasing Amounts of β-oxybutyric Acid, Acetone and Aceto-acetic Ethyl Ester on the Determination of Creatinine in Urine.

Concentration in urine, per cent.	Grammes per day in 1500 c.c. urine.	Scale reading in mm.	Creatinine, grm. per 100 c.c.	Error in the determination of creatinine, grm. per 100 c.c.
β-oxybutyric acid added to urine.				
0	0	7	0·116	
0·036	0·54	7	0·116	0
1	15·0	6·88	0·118	+0·002
2·16	32·4	7·27	0·111	−0·005
Acetone added to urine.				
0	0	7	0·116	
0·04	0·6	7·1	0·114	+0·002
0·17	2·5	7	0·116	0
1	15	7·6	0·106	−0·01
1·6	24	8·24	0·098	−0·018
7·6		12·34	0·066	−0·05
Aceto-acetic ethyl ester added to urine.				
0	0	7	0·116	
0·1	1·5	7·2	0·113	−0·003
0·5	7·5	7·5	0·108	−0·008
0·75	10·7	8·17	0·099	−0·017
1	15	6·9	0·117	+0·001
2	30	6·3	0·128	+0·012

Acetone if added to the urine in amounts less than 0·2 per cent. (Table I) does not introduce any error at all. A 1-per-cent. solution makes the colour lighter than usual, while if the acetone is present in larger amounts the colour becomes much lighter and it fades very rapidly on standing. As acetone is excreted in urine in very small amounts the creatinine determinations will not be affected, as a 0·17-per-cent. solution caused no error. These results do not agree with those of van Hoogenhuyze and Verploegh (13) and Krause (16), who found that a 1-per-cent. acetone solution made the colour darker, but that the error disappeared on standing.

Aceto-acetic ethyl ester when present in small quantities produces a slight lightening of the colour. Thus a 0·1- and 0·75-per-cent. solution causes an error in the scale reading of 0·2 and 1·1 mm. Larger amounts, on the other hand, cause a darkening effect, but the colour becomes much redder than usual, which makes it really impossible to match it with the N/2 bichromate solution. These results agree with those obtained by Krause (16), Wolf and Osterberg (22), and Rose (20). This experiment is not of much practical importance, as the ethyl ester is never excreted in urine, but we have made it because other observers have added this substance to urine instead of aceto-acetic acid.

The sodium salt of aceto-acetic acid produces a much more marked effect than the other acetone bodies. Even when added to urine in small amounts the colour obtained with picric acid and soda is not darker, as stated by Krause (16) and others, but is actually lighter, and when present in large amounts the colour is very much lighter (Table II and fig. 1). The error is not eliminated on standing but increases.

Table II shows the Effect of Increasing Amounts of Aceto-acetic Acid on the Estimation of Creatinine in Urine.

Aceto-acetic acid added to urine.		Scale reading.	Creatinine.	Error in the creatinine determination.	
grm. per 100 c.c.	grm. per 24 hrs. in 1500 c.c.		grm. per 100 c.c.	grm. per 100 c.c.	per cent.
0	0	7	0·116	0	
0·0234	0·35	7·33	0·111	0·005	4·3
0·0468	0·702	8	0·101	0·015	12·9
0·093	1·4	9	0·09	0·026	22·2
0·187	2·8	11·3	0·072	0·044	38
0·374	5·6	14·66	0·055	0·061	52·6

Thus if the concentration of the sodium aceto-acetate is only 0·0234 or 0·0468 per cent. the creatinine estimation is too low, the actual errors being 0·005 and 0·015 grm. respectively. The error produced by larger amounts is very striking, for if the concentration is increased to 0·374 per cent. the error is as great as 0·061 grm., and the percentage error in this case is 52·6 per cent. As amounts of aceto-acetic acid up to a concentration of 0·4 per cent. may be excreted in diabetes, the error caused in such cases must be very great. If the error in the creatinine determination be plotted against the concentration of the aceto-acetic acid the resulting curve is almost a straight line (fig. 1).

The chemistry of this action is at present engaging our attention.

It was not possible to isolate the pure acid and add it to urine, but this does not matter, as the aceto-acetic acid is excreted in the urine partly as the free acid and partly as a salt. Moreover, in the process of estimating the creatinine an excess of caustic soda is added, and this must convert all the free acid into the sodium salt.

The solution of sodium aceto-acetate used in our experiments also contained 0·37 grm. ethyl alcohol to 1 grm. of aceto-acetic acid.

The presence of ethyl alcohol in the sodium aceto-acetate solution (due to its mode of preparation from aceto-acetic ethyl ester) is a possible disturbing factor, as it might be the cause of the colour change. However, the addition of alcohol to urine in amounts which correspond to those added with the sodium aceto-acetate did not produce any alteration at all in the colour. The possibility still remained that it was the mixture of the alcohol with the sodium aceto-acetate which was responsible for the change in colour. There is no means of directly disproving this hypothesis, as the alcohol cannot be removed from the sodium aceto-acetate solution without destroying the salt. There is, however, indirect proof that this is not the case, as will be shown in the following paragraphs.

Aceto-acetic acid is excreted in the urine during carbohydrate starvation. As will be shown later on, the aceto-acetic acid can easily be removed from the urine without breaking down creatinine or creatine, and this procedure was followed in three diet experiments which will be described in detail later on. The urine which contained aceto-acetic acid had apparently less creatinine in it than the urine from which the aceto-acetic acid had been removed. Thus on the 2nd day Experiment I, a concentration of aceto-acetic acid of 0·065 per cent., caused an error in the creatinine figure of 0·017 grm. per 100 c.c., and a concentration of aceto-acetic acid of 0·081 per cent. on the third day caused an error in the creatine of 0·028 grm. per 100 c.c. On the second and third days of Experiment II the concentration of aceto-acetic acid of 0·085 and 0·112 per cent. produced errors of 0·018 and 0·027 grm. per 100 c.c. respectively (Table III).

Table III shows the Error caused in the Estimation of Creatinine caused by the Excretion of Aceto-acetic Acid in the Urine consequent on Carbohydrate Starvation. (Extracted from Tables VII–IX, pp. 217 and 218, of this paper.)

Day.	Concentration of aceto-acetic acid per 100 c.c.	Error caused by aceto-acetic acid in determination of creatinine.
		grm. per 100 c.c.
Diet, Experiment I—		
2	0·065	0·017
3	0·081	0·028
Diet, Experiment II—		
1	0·029	0·005
2	0·085	0·018
3	0·112	0·027
Diet, Experiment III—		
1	0·036	0·01
2	0·072	0·008

These figures have been plotted on the fig. 1 previously referred to and they lie fairly close to the curve. The figures for the second day of Experiment I and for both days of Experiment II lie somewhat below the curve, while the figure for the third day of Experiment I lies a little above the curve, but the difference is in no case great.

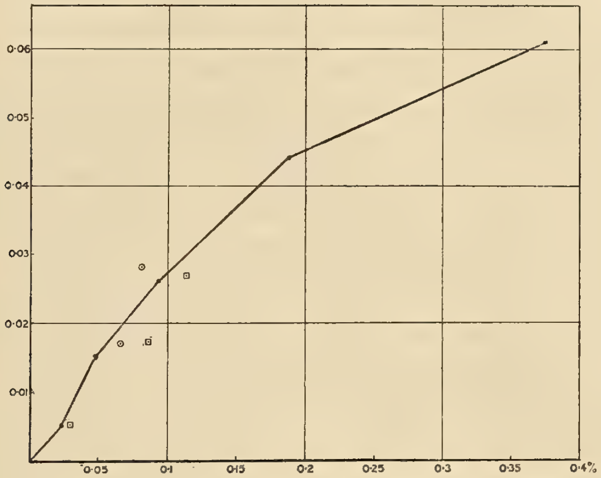

Fig. 1.—The curve shows the error in grammes per 100 c.c. in the estimation of creatinine caused by increasing amounts of aceto-acetic acid. Abscissæ: percentage concentrations of aceto-acetic acid. Ordinates: the error in the creatinine determinations expressed in grammes per 100 c.c. ⊙ = the error in the creatinine determinations on second and third days of Expt. I (Table VII). ⊡ = the error on first, second, and third days of Expt. II (Table VIII).

It must be remembered that the figure for the concentration of the aceto-acetic acid in the diet experiments was obtained by the Messinger-Huppert method, which makes no distinction between acetone and aceto-acetic acid. It is true that acetone is only present in urine in small amounts, but in considering the effect of the aceto-acetic acid some allowance should be made for the amount of acetone present. A diminution in the concentration of the aceto-acetic acid would make the points below the curve more nearly approximate to the curve but would displace the point above the curve away from the curve.

The general agreement between the errors produced by aceto-acetic acid in these experiments, and the errors produced when sodium aceto-acetate containing ethyl alcohol is added to urine, point to the conclusion that it is the aceto-acetic acid itself and not the alcohol that causes the errors, in the latter case.

Our experiments agree with those of Greenwald and show that the aceto-acetic acid produces a considerable error in the estimation of creatinine, so that the result obtained is too low.

II. *A Method for Removing Aceto-acetic Acid from Urine Preliminary to the Estimation of Creatinine.*

In Folin's method for estimating the creatinine + creatine, the creatine is converted into creatinine by heating on a water-bath for three hours with normal hydrochloric acid. This procedure removes all the aceto-acetic acid from the urine by converting it into acetone, which is distilled away. Thus the aceto-acetic acid could not be detected by Rothera's (21)* nitroprusside test after one hour's heating. Consequently the estimation of the creatinine + creatine will not be disturbed by the presence of any aceto-acetic acid and will be accurate, but the result of the creatinine estimation which is carried out in the presence of aceto-acetic acid will be too low. Consequently the result obtained for the creatinine + creatine will be higher than that for the creatinine and will lead to the conclusion that creatine is present in the urine whether this is actually the case or not.

The aceto-acetic acid must, therefore, be removed from the urine before the determinations are made. Greenwald (12) extracted the urine with ether for two hours and found that the aceto-acetic acid was all removed by that process; the ether was subsequently removed by aëration for one hour. This process involved some dilution of the urine and in order to get over this difficulty Greenwald added twice the amount of picric acid and soda. This method takes some time to carry out and in our experience it is better if possible to avoid all dilution of the urine, especially when the urine is dilute to begin with, in order to get correct results.

The method which we employed (10) is very much simpler and we have now tested it carefully and modified it slightly (11).

1 c.c. of 10-per-cent. phosphoric acid is added to 10 c.c. of urine in a boiling tube 200 mm. long and 30 mm. wide. The mixture is then heated in a water-bath of which the temperature is between 65° and 70° C. and at a pressure of about 210 mm. of mercury produced by means of a filter pump. Bumping is prevented by allowing air to bubble slowly through the liquid by means of a capillary tube dipping into it. The temperature must not rise above 70° C. nor the pressure fall below 210 mm. or else concentration of the urine takes place. If the above directions are followed only a few drops of liquid are distilled over into the receiver, as the result of three-quarters of an hour's distillation. At the end of this time the process is stopped and the solution

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* Rothera's test has been shown by Hurtley (14) to be a test for aceto-acetic acid as well as for acetone.

cooled. The mixture in the boiling tube is neutralised with 1·5 c.c. of 10-per-cent. soda* and then 15 c.c. of saturated picric acid and 5 c.c. of the soda are added. The mixture is allowed to stand seven minutes and then the contents of the boiling tube are washed into a 500 c.c. flask and diluted up to 500 c.c. with water. By heating for three-quarters of an hour, the aceto-acetic acid can be completely removed even if it is present in a concentration of 0·2 per cent.

If the concentration of aceto-acetic acid is greater than 0·2 per cent., the distillation must be continued for a longer time, and the complete removal of the aceto-acetic acid must be ascertained by testing a control with Rothera's nitroprusside test. We have continued the distillation for one and a half hours, and find that no error in the creatine estimation occurred.

We have tested the method in the following manner. The amount of creatinine in a normal urine was determined, and aceto-acetic acid was then added in varying amounts to the urine, and the creatinine again estimated. The aceto-acetic acid was then removed by the distillation method, and the creatinine again estimated. The results obtained show that the distillation did not break up any of the creatinine, and that the aceto-acetic acid was completely removed (Table IV).

Table IV shows that the Error caused by Varying Concentrations of Aceto-acetic Acid in the Urine is completely removed by the Distillation Method.

Amount of aceto-acetic acid added to urine.	Creatinine by Folin's method.		Creatinine after removal of the aceto-acetic acid by the distillation method.
	Urine alone.	Urine + aceto-acetic acid.	
grm. per 100 c.c.	grm. per 100 c.c.	grm. per 100 c.c.	grm. per 100 c.c.
0·088 approx.	0·145	0·128	0·145
0·044 ,,	0·108	0·097	0·105
0·18	0·096	Not estimated	0·096
0·27	0·096	0·053	0·094

One of the most important questions to decide was whether creatine was converted into creatinine in this process of distillation.

Pure crystalline creatine† was added to normal urine in varying amounts, and the creatinine was then determined by Folin's method and by the distillation method. The creatine was then converted into creatinine by heating on the water-bath for three and a half hours or longer with 5 c.c. normal hydrochloric acid, in order to get the creatinine + creatine figure. These experiments show that in no case was any creatine converted into creatinine by the distillation, even when (Experiment 5) there was actually more creatine (0·152 grm.) than creatinine (0·119 grm.) in the urine (Table V). In the first three experiments, when creatine was present in small amounts, practically all the creatine added to the urine was converted into creatinine by heating for three and a half hours on the water-bath. In the fourth experiment, when 0·076 grm. of creatine was present in the urine, only 48 per cent. of the creatine was converted into creatinine after three and a half hours, and even after five and a half hours only 89 per cent. could be recovered. In the fifth experiment, with a very large amount of creatine (0·152 grm. per 100 c.c.), only 28 per cent. was converted after heating for three and a half hours.

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* The phosphoric acid must be adjusted against the 10-per-cent. caustic soda, and the correct amount of caustic soda necessary to neutralise 1 c.c. phosphoric acid must be added.

† We wish to thank Dr. F. G. Hopkins and Mr. Mackenzie Wallis for kindly supplying us with the pure creatine.

Table V shows that Creatine is not broken down into Creatinine by Heating with Phosphoric Acid in the Distillation Method.

No. of expt.	Amount of creatine calculated as creatinine added to urine.	Creatinine.		Creatinine + creatine calculated as creatinine.	Creatine recovered in the estimation as creatinine.	Creatine added to urine as creatinine.	Per-centage recovered.
		Folin's method.	Distillation method.				
	grm. per 100 c.c.	grm. per 100 c.c.	grm. per 100 c.c.	grm. per 100 c.c.	grm. per 100 c.c.	grm. per 100 c.c.	
1	0·01	0·071	0·071	0·081†	0·01	0·01	100
2	0·016	0·095	0·096	0·110†	0·015	0·016	95
3	0·034	0·112	0·113*	0·145†	0·033	0·034	97
4	0·076	0·117	0·117	0·154† 0·185‡ 0·183§	0·037† 0·068‡ 0·063§	0·076	47·5 89·5 83
5	0·152	0·119	0·120	0·163†	0·044	0·152	24

* Distilled 1½ hours.
† Heated for 3½ hours on the water-bath.
‡ ,, 5½ ,, ,,
§ By the autoclave method.

Benedict and Myers (3) have also noticed that the water-bath method gave an incomplete result. when urine containing 0·066 grm. creatine per 100 c.c. was used. Mellanby (18) has pointed out that five hours' heating on the water-bath is usually necessary to get a complete conversion. However, our observations show that three and a half hours on the water-bath is sufficient to estimate accurately small amounts of creatine up to 0·034 grm. per 100 c.c., but that a greater quantity than this cannot be converted quantitatively into creatinine in three and a half hours. Larger amounts than 0·034 grm. per 100 c.c. must be heated for more than three and a half hours, and it is very difficult, even so, to convert all the creatine into creatinine. As the greatest amount of creatine found by Cathcart (5) in a case of carbohydrate starvation was 0·38 grm. per day, three and a half hours' heating on a water-bath would be quite sufficient to convert practically all the creatine, that might be present in the urine, into creatinine.

Finally, to test the accuracy of the distillation method, we have employed it for urines containing both creatine and aceto-acetic acid in solution together (Table VI). In one experiment creatine was added to normal urine, and the estimations were carried out with and without the addition of aceto-acetic acid. In the second experiment the creatine was added to part of the urine of the third day of the diet experiment (Table VIII). In this case the urine already contained aceto-acetic acid. The results (Table VI) show that, even when both aceto-acetic acid and creatine are present at the same time, the aceto-acetic acid can be removed from the urine without breaking up the creatine, and that the creatine, if present in small amount, can be converted almost quantitatively into creatinine after three and a half hours' heating. The series of control experiments shows that the distillation method gives satisfactory results.

Table VI shows the Accuracy of the Distillation Method for Urines containing both Creatine and Aceto-acetic Acid.

		Creatinine.		Creatinine + creatine (as creatinine), Folin's method.	Creatine recovered by experiment (as creatinine).	Percentage recovered.
		Folin's method.	Distillation method.			
		grm. per 100 c.c.	grm per 100 c.c.	grm. per 100 c.c.	grm. per 100 c.c.	
A	Normal urine + creatine 0·01 grm. per 100 c.c.	0·071	0·070	0·080	0·01	100
	Same urine + creatine 0·01 grm. per 100 c.c. + 0·06 grm. of aceto-acetic acid per 100 c.c.	0·047	0·070	0·079	0·009	90
B	Urine of Day 3, Experiment II, containing 0·112 grm. of aceto-acetic acid per 100 c.c.	0·10	0·119	0·119	0	
	Same urine + creatine 0·04 grm. per 100 c.c.	0·10	0·120	0·156	0·036	90

In the examination of any urine which contains aceto-acetic acid, and which is thought to contain creatine, two estimations are required, namely, that of the total creatinine + creatine by the original Folin method, and that of the creatinine alone by the method given in this paper.

III. *The Alleged Excretion of Creatine on a Carbohydrate-free Diet.*

It is well known that the consumption of a diet containing no carbohydrates produces acidosis, with the excretion of β-oxybutyric acid, aceto-acetic acid, and acetone. The aceto-acetic acid will cause an error in estimating creatinine and creatine, and must be removed to get accurate results.

We have performed three diet experiments on three separate individuals, and have investigated the creatinine and creatine excretion, taking this precaution. The experiments were begun about 12 hours after the last ordinary meal in Experiments I and III, and six hours after, in Experiment II. Tables VII, VIII, and IX show the various determinations made. The creatinine was first of all estimated directly by Folin's method without removing the aceto-acetic acid, and the results are referred to as "apparent creatinine." The true creatinine was then obtained after removing the aceto-acetic acid by the distillation method. The creatinine + creatine was determined by heating the urine for three and a half hours on the water-bath with hydrochloric acid. By subtracting the apparent creatinine values from the creatinine + creatine output, the apparent creatine was obtained, and, by subtracting the true creatinine from the creatinine + creatine, the true creatine output was obtained. Duplicate determinations were performed in each case, and in each determination the mean of six to eight readings of the scale was taken.

In Experiment I (E. P. P.), cream alone was eaten on the first two days; on the third day protein was added to the diet. The calorie value of the diet was low. The effect of the withdrawal of carbohydrates was shown by the prompt appearance of aceto-acetic acid in the urine. On the first day the nitroprusside reaction (Rothera's) was faint, but on the second and third days it was well marked, and 0·872 and 0·874 grm. of aceto-acetic acid were excreted. On the first day the apparent creatinine was 1·82 grm., while the true creatinine and creatinine + creatine was 1·78 and 1·80 respectively, so that no creatine was excreted in the urine, as the difference is within the limits of experimental error. On the 2nd day the apparent creatinine was diminished to 1·58 grm., while the true creatinine and creatinine + creatine were practically the same as on the previous day, *i.e.*, 1·81 and 1·82 grm. The apparent creatine was, therefore, 0·24 grm., while no true creatine was excreted. On the third day the apparent creatinine had fallen to 1·42 grm., while the true creatinine and creatinine + creatine was still 1·72 grm. The apparent creatine had, therefore, increased to 0·31 grm., while as a matter of fact no true creatine was excreted.

In Experiment II (G. G.) ¾ pint of cream and two eggs were eaten on each day. The amount of aceto-acetic acid excreted was greater than in Experiment I, and the nitroprusside reaction was quite strong on the first day, 0·3 grm. being excreted. On the second and third days the aceto-acetic acid amounted to 1·06 and 1·46 grm. The true creatinine output was slightly lower than in the case of E. P. P., but it remained equally constant

Table VII.—Experiment I. Subject, E. P. P. Date, June 18–20, 1913.

Day.	Volume.	Total nitrogen.	By the Folin method.				After removal of aceto-acetic acid.		Aceto-acetic acid.	
			Apparent creatinine.	Creatinine + creatine.	Apparent creatine.		True creatinine.	True creatine.	Grm. per day.	Concentration in 100 c.c.
					Grm. per day.	Grm. per 100 c.c.	Grm. per day.	Grm. per day.		
	c.c.	grm.	grm.	grm.						
1	740	12	1·82	1·80	0	0	1·78	0	—	—
2	670	13·5	1·58	1·82	0·24	0·018*	1·81	0	0·872	0·065*
3	1070	16·25	1·42	1·72	0·30	0·028	1·72	0	0·874	0·081

Diet eaten.—Day 1 and 2: Cream, 300 c.c. Calorie value (approximate), 1060.
Day 3: Cream, 300 c.c.; plasmon, 50 grm.; eggs, 2. Calorie value (approximate), 1640.

* On this day the volume of urine was small and an equal volume of water was added to it before the creatinine determinations were made in order to get a reading on the colorimeter scale within the limits advised by Folin. This dilution will halve the concentration of the aceto-acetic acid in the solution used for the Folin estimation, which becomes actually less than that of the succeeding day.

Table VIII.—Experiment II. Subject, G. G. Date, June 22–25, 1913.

Day.	Volume.	Total nitrogen.	By the Folin method.				After removal of aceto-acetic acid.		Aceto-acetic acid.	
			Apparent creatinine.	Creatinine + creatine.	Apparent creatine.		True creatinine.	True creatine.	Grm. per day.	Concentration per 100 c.c.
					Grm. per day.	Grm. per 100 c.c.				
	c.c.	grm.	grm.	grm.			grm.	grm.		
1	1310	—	1·46	1·53	0·07	0·0053	1·52	0	0·3	0·029
2	1235	12·4	1·21	1·43	0·22	0·0175	1·43	0	1·06	0·085
3	1310	11·86	1·17	1·53	0·36	0·0274	1·52	0	1·46	0·112

Diet eaten.—Cream, 400 c.c.; eggs, 2. Calorie value (approximate), 1400.

Table IX.—Experiment III. Subject, M. D. Date, June 25–28, 1913.

Day.	Volume.	Total nitrogen.	By the Folin method.				After removal of aceto-acetic acid.		Aceto-acetic acid.	
			Apparent creatinine.	Creatinine + creatine.	Apparent creatine.		True creatinine.	True creatine.	Grm. per day.	Concentration per 100 c.c.
					Grm. per day.	Grm. per 100 c.c.				
	c.c.	grm.	grm.	grm.						
1	600	11·99	1·93	2·05	0·12	0·010	2·03	0	0·43	0·036*
2	750	14·43	2·02	2·14	0·12	0·008	2·15	0	0·99	0·066*
3	1220	14·82	2·09	2·25	0·16	—	2·27	0	—	—

Diet eaten.—Cream, 500 c.c.; eggs, 3. Calorie value (approximate), 1600 calories.

* On these days the volume of urine was small and an equal volume of water was added to it before the creatinine determinations were made in order to get a reading on the colorimeter scale within the limits advised by Folin. This dilution will halve the concentration of the aceto-acetic acid in the urine.

throughout the experiment, viz., 1·5 grm. On the first day the apparent creatine was already 0·07 grm., and on the second and third days it had risen to 0·22 and 0·36 grm. respectively, but no true creatine was excreted at all. The difference in the scale reading between the apparent and true creatinine was 2 mm. on the third day of this experiment. It has been previously shown (p. 211, fig. 1) that the error in the estimation of the creatinine caused by the aceto-acetic acid in these two experiments agrees fairly closely with the error caused by adding the same concentration of a sodium aceto-acetate solution to normal urine.

As it was necessary to be absolutely certain that if any creatine was present in the urine it would be converted into creatinine by the methods we have used, some pure creatine was added to a part of the urine of the third day in Experiment II. The estimation of the creatinine + creatine in the plain urine and in the urine to which creatine had been added was carried out under precisely similar conditions on the same water-bath. The result (Table VI) showed that in the plain urine no creatine was converted into creatinine, but that the creatine was almost quantitatively converted into creatinine in the sample of urine to which creatine had been added. This control experiment shows that creatine if present in the urine is detected and estimated by the methods employed.

As we wished to confirm the results of these experiments on ourselves, Dr. M. Donaldson very kindly took the following diet for three days, viz., ¾ pint of cream and three eggs each day. We wish to express our thanks to him. The urine gave Rothera's nitroprusside test on the first day, and this reaction was well marked on the second and third days. The true creatinine in the urine was again very constant for the three days, lying between 2 and 2·3 grm. On the first and second days the apparent creatine was 0·12 grm., and on the third day it was 0·16, while no true creatine was excreted. The apparent creatinine was not so low as in Experiments I and II, but its difference from the true creatinine was quite definite enough to be measured on the colorimeter.

Discussion of Results.

These three experiments show that the removal of carbohydrate from the diet causes an excretion of aceto-acetic acid in sufficient amount to cause an error in the estimation of creatinine, so that the results are too low and creatine is apparently excreted.

Cathcart (5), Benedict (2), Mendel and Rose (19) state that creatine occurs in the urine under somewhat similar conditions to those under which we have worked. The amounts of creatine they obtained were about the same as those apparently obtained by us, before we removed the aceto-acetic acid, *e.g.* the largest amount that Cathcart (5) found on a fat diet was 0·38 grm., which is slightly more than the apparent creatine we found on the third day of Experiment II.

Cathcart remarks that the creatinine excretion diminishes to a certain extent, as the result of a fat diet. We have found that it remains constant throughout, and in the case of G. G. agreed very closely with the amount of creatinine excreted 15 months before on a pure fat and carbohydrate diet (10). However, the error caused by the presence of aceto-acetic acid in the urine results in less creatinine being found than is actually present.

Our experiments extended over about the same time as those of Cathcart, but there was a slight difference, viz., that we had no preliminary starvation day. However in Experiment I the condition of semi-starvation was really very similar to that of one day's complete starvation, as only half a pint of cream was taken and the calorie value was 1060.

We have also published a case of carbohydrate starvation (10) lasting for 10 days in which the diet had a calorie value of only 1969 per diem. No creatine was excreted at any time, and the creatinine excretion remained constant throughout.

From these results we draw the conclusion that mere carbohydrate starvation itself does not cause an excretion of creatine in the urine.

Naturally, no conclusion can be drawn from these experiments as to whether creatine is excreted during prolonged periods of total starvation, but we maintain that in all those many physiological and pathological conditions in which acetone bodies are excreted in the urine, the estimations of creatinine and creatine must be inaccurate, unless the precaution is taken of removing the aceto-acetic acid from the urine.

Conclusions.

1. The presence of aceto-acetic acid always causes an error in the estimation of creatinine and the error increases with increasing amounts of aceto-acetic acid. As the result of this error the estimation of creatinine will be too low. This error is not eliminated if the diluted urine is allowed to stand for varying lengths of time before making the readings.

2. The aceto-acetic acid is removed in the estimation of creatinine + creatine and does not cause any error.

3. As the creatinine figure is too low and the creatinine + creatine figure is correct, it will appear that creatine has been excreted.

4. Acetone and β-oxybutyric acid, if present in amounts comparable to those which usually occur in urine, produce practically no error in the estimation of creatinine.

5. A simple and reliable method has been devised for removing aceto-acetic acid, preliminary to the estimation of creatinine.

6. In our experiments a carbohydrate-free diet did not cause the excretion of any creatine.

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On Medullosa pusilla.

By D. H. Scott, LL.D., D.Sc., For.Sec.R.S.

(Received August 12,—Read November 20, 1913.)

[Plate 13.]

In the second edition of my 'Studies in Fossil Botany,' I referred in the following words to the fossil plant which forms the subject of this notice. "A very small *Medullosa* (named provisionally *Medullosa pusilla*), the stem with the leaf-bases not exceeding 2 cm. in diameter, has since been found by Mr. P. Whalley, of Colne, Lancashire. The stem has three steles, and agrees very closely with *M. anglica*, except in size."*

In order to clear the ground for other observers, it now seems desirable to give some further account of this fossil, with the necessary illustrations. Though the plant differs in no important respect from the now well-known species *M. anglica*, it is of some interest, as probably the smallest *Medullosa* on record.

The fossil, as Mr. Whalley informs me, comes from the Soap-stone, immediately above the Halifax Hard Bed of the Lower Coal Measures. Its horizon may thus be compared with that of the roof-nodule specimens in other localities.

I have only two sections of the stem, sent me by Mr. Whalley on January 24, 1906. There are also a couple of sections received this year which appear to be of the same plant, and perhaps of the same specimen, but only show a leaf-base or petiole.

Both the sections of the stem are transverse, but in one of them a stele is shown partly in longitudinal section, owing, no doubt, to displacement (Plate 13, fig. 2, above). This has enabled me to compare the minute structure of the wood with that of *M. anglica*.

General Structure.

The extreme dimensions of the specimen as shown in the sections are 22 × 13 mm.; the form is much distorted, and in the direction of the longest diameter tissue has manifestly been lost. Three leaf-bases are present, one of which is well preserved and practically complete, while the other two are crushed and imperfect (figs. 1 and 2). They all contain numerous vascular bundles, and are bounded by a "Sparganum" cortex,

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* P. 441, footnote.

The best leaf-base measures about 13 mm. in the tangential and 8 mm. in the radial direction.

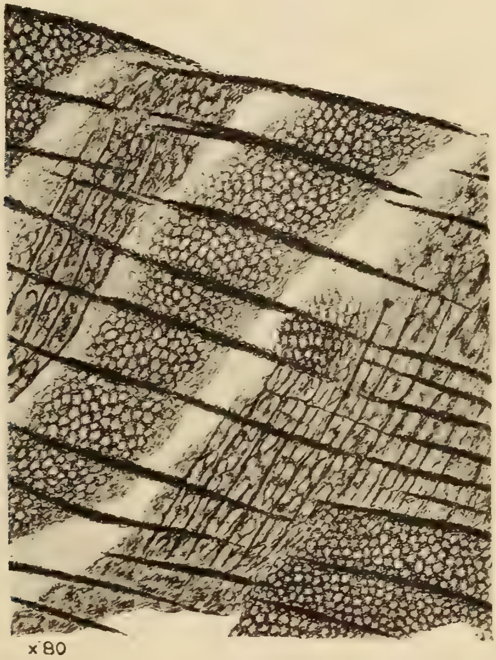
The tri-stelar vascular system of the stem (fig. 3) is enclosed in a definite but irregular ring of dark tissue, which, judging from the best-preserved portion, is evidently an internal periderm (*cf.* text-fig. B). The approximate dimensions of the region enclosed by the periderm are 7 × 5·5 mm. The general structure is clearly the same as that of *M. anglica,* in which three leaf-bases appear in the transverse section, and the vascular system is also normally tri-stelar (Scott, '99, text-fig., p. 126, Plate 5, Phot. 1). In the specimen of *M. anglica* referred to, the dimensions in its present condition are 10·5 × 3·7 cm. The natural diameter would no doubt have been a little over 7 cm., and the other specimens investigated do not differ greatly in size. In *M. pusilla* the natural diameter cannot be directly measured, as two of the leaf-bases are crushed and incomplete. Judging from the radius of the best-preserved portion, the true diameter must have been just about 2 cm. This gives a proportion between *M. anglica* and *M. pusilla* of rather more than 3·5 : 1. If we compare the stelar systems, the difference is somewhat greater—that of *M. anglica* in the best-preserved specimen measuring about 4 × 2 cm. as against 7 × 5·5 mm. in *M. pusilla,* taking the periderm as the boundary in both cases. From the means, 3 cm. and 0·625 cm. respectively, we get a proportion of 4·8 : 1. Roughly, we may say that the linear dimensions of *M. pusilla* were about one quarter of those of a typical specimen of *M. anglica.*

Stelar System.

The three steles are nearly equal in size, attaining a diameter of about 3 mm. Little is preserved except the wood, though here and there remains of the cambium and phloem can be found. The primary wood has a somewhat triangular transverse section (fig. 3). It is composed for the most part of tracheides, with comparatively little xylem-parenchyma among them. The smallest elements, presumably protoxylem, are found at the prominent angles, very near the outside of the primary xylem, but whether the structure was slightly mesarch or actually exarch could not be determined with certainty; there is evidence pointing in both directions. Similar difficulties were met with in the case of *M. anglica,* but there, with the help of the longitudinal sections, it was possible to obtain definite proof of mesarch structure (Scott, '99, Plate 10, fig. 5). In *M. pusilla* the partially longitudinal section of one stele does not clearly show the position of any protoxylem group.

The secondary wood is very unequally developed, attaining its greatest thickness, about 12 elements, on the inner side of the steles (fig. 3), as has also been observed in *M. anglica* (Scott, '99, p. 89, Plate 5, Phot. 1, Plate 6, Phot. 5). On the outer side it thins out, or may even be interrupted, perhaps in connection with the departure of a leaf-trace bundle. The medullary rays are numerous, with the tracheide-bands between them only 1–3 elements in width.

The longitudinal section shows something of the primary as well as the secondary wood. In both, the tracheides have multiseriate bordered pits, sometimes ranged in as many as six rows. One or two narrow scalariform or spiral elements can also be recognised in the outer part of the primary xylem. The secondary wood is cut approximately in the radial direction, and several medullary rays are shown; they are muriform, with very low cells; the pits adjacent to the ray-cells are elongated radially (text-fig. A). The wood is in all respects similar to that of *M. anglica.* Only one leaf-trace bundle is shown in connection with the stelar system, and in an undivided condition (fig. 3, *l.t.*). It measures about 650 × 570 μ. There is no obvious secondary wood, and the smallest elements appear to be directed outwards, but the preservation is imperfect.



TEXT-FIG. A.—Approximately radial section of part of secondary wood, showing tracheides with multiseriate bordered pits and muriform medullary rays. Drawn by Mr. G. T. Gwilliam. × about 80. Scott Coll. 2818.

Except for the possible absence of secondary wood from the undivided leaf-trace—a doubtful point on which no stress can be laid—there is no difference between the stelar system of *M. pusilla* and that of *M. anglica.*

The Cortex and Leaf-bases.

In *M. pusilla,* as in *M. anglica,* no sharp limit can be drawn between cortex and leaf-base except at a level where the latter is already marked off by an internal barrier of sclerotic strands. The appearance of such a barrier is, of course, a preparation for the departure of the leaf-base from the stem.

In the transverse sections the best preserved leaf-base is only partly delimited in this way; a sclerotic band runs inwards from each side, but does not extend right across. Of the two imperfect leaf-bases, one appears to be completely marked off by an internal sclerotic band, while the other is not yet delimited at all (Plate 13, figs. 1 and 2)

A convenient boundary between cortex and stelar system is provided by the periderm (fig. 3). The cortex, which contains numerous gum-canals, is not very well preserved, but it can be seen that the vascular bundles in this region are, on the whole, larger and rounder in section than those which have definitively entered the leaf-base.

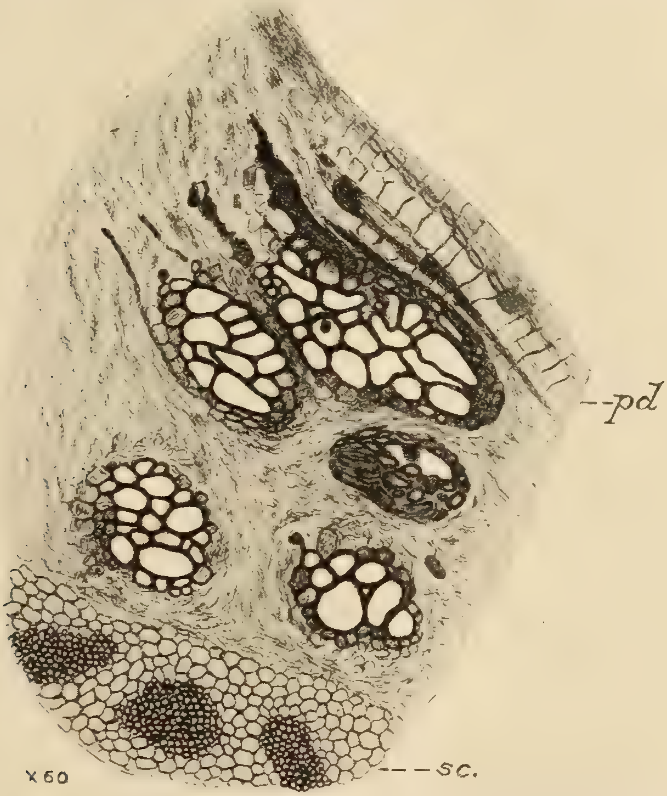
At one place a group of four or five bundles is shown, which has, to all appearance, arisen from the division of a single primary leaf-trace (text-fig. B). This group lies in the cortex, which is here well defined by the sclerotic band on the exterior and the periderm within. Similar groups of bundles formed by division are well known in the cortex of *M. anglica* (Scott, '99, Plate 6, Phot. 9; Plate 11, fig. 12). In both species later stages in the division of the bundles are found in the leaf-base itself.

The well-preserved leaf-base is best shown in the section represented in Plate 13, fig. 2, and is here sufficiently perfect for the bundles to be counted with approximate accuracy. There are 15 peripheral bundles (*i.e.* on the free side), nine on the side attached to the stem, and eight, of which two are double, in an intermediate position. Thus the interior of the leaf-base is poor in bundles, a condition which also exists in that of *M. anglica* at a corresponding level (Scott, '99, p. 100).

The bundles near the periphery have assumed their definitive petiolar character, while the inner bundles more resemble those of the cortex, and are still undergoing division. Some of the former are very well preserved (Plate 13, fig. 4), with the phloem practically perfect; the usual exarch, collateral structure is obvious. The sectional form of these bundles is often slender, *i.e.* elongated radially, as also occurs sometimes in *M. anglica,* Thick-walled elements enclose the xylem of the bundle in an interrupted, hippocrepiform sheath, but do not extend round the phloem.

The parenchyma contains some gum-canals and presents no peculiarities.

The only differences between the leaf-base of *M. pusilla* and that of *M. anglica* are in the hypoderma. This zone is rather narrow in *M. pusilla*, its usual thickness being about 400 μ; in *M. anglica* it ranges from 2 to 3 mm., *i.e.* it is about six times as thick, on the average, while the general dimensions are only about four times as great. Further, in *M. pusilla* the hypoderma is much simpler ; as a rule, the sclerotic strands are in a single rank, and they never stand more than two deep (Plate 13, fig. 4). In the leaf-base of *M. anglica* the strands are three to four deep (Scott, '99, Plate 5, Phot. 3; Plate 12, fig. 14). In *M. pusilla* the principal strands are about twice as deep as they are wide, and slightly wedge-shaped, widening



Text-fig. B.—Group of vascular bundles from the cortex, probably resulting from the division of a single leaf-trace ; *pd.* periderm, marking inner limit of cortex ; *sc.*, internal sclerotic band, marking inner limit of leaf-base. Drawn by Mr. G. T. Gwilliam. × about 60. Scott Coll. 2818.

outwards. In *M. anglica*, where they are so much more numerous, they are quite irregular in form.

The distinction is not absolute, for in parts of the rachis attributed to *M. anglica* one may find much the same arrangement as in *M. pusilla*. Comparing leaf-base with leaf-base, however, there appears to be a real difference between the two plants. The hypoderma of *M. pusilla* is of the type of Renault's *Myelopteris Landriotii*, var. α (Renault, '75, Plate 5, fig. 41), while that of *M. anglica* is more like his var. β (*loc. cit.*, Plate 4, fig. 29). Of course, the agreement is far from exact, for Renault's petioles no doubt belonged to quite different species of *Medullosa* from ours.

In the leaf-base of *M. pusilla* there are very few gum-canals visible in the hypoderma, and they are not very numerous altogether (fig. 4). In *M. anglica* they are very common in the hypoderma, and fairly so elsewhere. This distinction, however, is of very doubtful value, for in a section of a detached petiole or leaf-base probably referable to *M. pusilla*, gum-canals are numerous in the parenchyma outside the sclerotic strands, most of the strands having canals corresponding to them. This tissue is hardly preserved at all in the type-specimen, so the small number of hypodermal canals observed may be deceptive. Their distribution appears to have been a little different from that in *M. anglica*, but in that species also the external parenchyma is seldom well preserved, so comparison is difficult.

Summary and Conclusions.

If we draw up a diagnosis of *M. pusilla* on the lines of that given for *M. anglica* (Scott, '99, p. 111), we find few distinctions between them, apart from size.

Medullosa pusilla.—(Scott, 'Studies in Fossil Botany,' second edition, p. 441, 1909.)

Stem clothed by the relatively large decurrent leaf-bases.

Vascular system of stem consisting of three uniform steles. Star-rings absent. Interior of each stele wholly occupied by primary wood.

Secondary wood of moderate thickness, most developed on the inner sides of the steles. Tracheides (apart from the protoxylem) with multiseriate bordered pits.

Leaf-traces probably concentric on leaving the steles, but *with little or no secondary wood*, branching and becoming collateral as they pass into the leaf-bases.

Leaf-bases, with a narrow hypoderma, consisting of a single, or locally double series of strands, and resembling that of Myelopteris Landriotii, *var.* α Renault.

Gum-canals numerous in the cortex, *scattered in the leaf-bases. Stem small, about* 2 *cm. in diameter, including the leaf-bases.*

Locality: Lark Hill Pit, Colne, Lancs. *Horizon:* Soap-stone, overlying Halifax Hard Bed, Lower Coal Measures.

Found by Mr. P. Whalley, 1906.

I have italicised the characters in which this form differs from *M. anglica.* The distinctions are of little importance, with the exception of the small size of the plant and the simpler structure of the hypoderma, points which appear to be of some diagnostic value.

The question arises whether it is worth while to separate the species from *M. anglica* on these somewhat slender grounds. The difference in size is considerable, and not due merely to age, for secondary growth is already fairly advanced, while the whole primary structure is on a small scale.

The specimen might, however, be from the basal part of the stem, where it had not reached its full dimensions, or might belong merely to a dwarfed plant. At the same time, it is perhaps equally probable that our specimen represents a distinct species. There is strong reason to believe that the foliage of *M. anglica* was of the *Alethopteris* type (Scott, '99, p. 102), and it is probable that the species may have been identical with *A. lonchitica,* so abundant in the Lower Coal Measures. We have no evidence as to the foliage of *M. pusilla,* but there is a certain presumption that it was also that of an *Alethopteris,* both from analogy with *M. anglica* and from the older observations of Renault. That author, after a careful comparison, came, as is well known, to the conclusion that it was extremely probable that the petioles of his *Myelopteris* (*Myeloxylon*) *Landriotii* bore the fronds of certain species of *Alethopteris* (Renault, '83, p. 165). In his 'Cours de Botanique Fossile' he reproduces the figure of *M. Landriotii,* var. α, under the title "Section Transversale d'un Pétiole d'*Alethopteris*" ('83, Plate 28, fig. 1). This is the section which most closely resembles the leaf-base of *M. pusilla.* If *M. pusilla* was the stem of an *Alethopteris* other than *A. lonchitica,* it may conceivably have belonged to the closely allied but somewhat smaller plant, *A. decurrens,* the only other species which appears to be frequent in the British Lower Coal Measures.* This is a mere conjecture, but, at any rate, it is reasonable to suppose that more than one *Medullosa* existed in our Lower Coal Measure flora, and, as our specimen is quite peculiar in its small dimensions, and has other slight distinctive characters, we may provisionally treat it as representing a new species.

My friend, Dr. Lotsy, has proposed to divide the genus *Medullosa* into two new genera, *Neuropteromedullosa* and *Pecopteromedullosa,* the former having a complex stelar system together with *Neuropteris* foliage, while the latter is characterised by a relative simple stelar system (as in *M. anglica*) and the foliage of *Alethopteris* (Pecopterideæ) (Lotsy, '09, p. 725.) If we adopted this division, the name of our fossil would, of course, become *Pecoptero-medullosa pusilla.*

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* Kidston, '93, pp. 225, 245; Franke, '12, p. 42.

The facts at present known are not, however, favourable to a division of the genus *Medullosa* on these lines. *M. Leuckarti,* one of the complex Permian species, bore, according to the observations of Weber and Sterzel ('96, p. 48 [89]), leaf-bases belonging to *Myeloxylon Landriotii* (Ren.), and its foliage was therefore presumably of the *Alethopteris* type. Uncertain as this conclusion may be, we clearly have no grounds for assuming that a highly differentiated stelar system was necessarily associated with *Neuropteris* foliage.

In the present state of our knowledge, it would only be possible to subdivide the genus *Medullosa* on purely anatomical characters, and at the present moment even this seems to me to be premature.

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EXPLANATION OF PLATE.

Figures are from microphotographs by Mr. W. Tams.

Fig. 1.—General transverse section of the stem and leaf-bases. × 3. Scott Coll. 2817.

Fig. 2.—The other transverse section (reversed, as compared with fig. 1). One of the three steles is destroyed and another displaced, so as to appear in nearly longitudinal section. One leaf-base is perfect. × 3. Scott Coll. 2818.

Fig. 3.—The stelar system from fig. 1; *pd.*, periderm; *l.t.*, leaf-trace. × 11½. Scott Coll. 2817.

Fig. 4.—Part of the leaf-base, showing the hypoderma and a bundle with xylem and phloem. × 45. Scott Coll. 2817.

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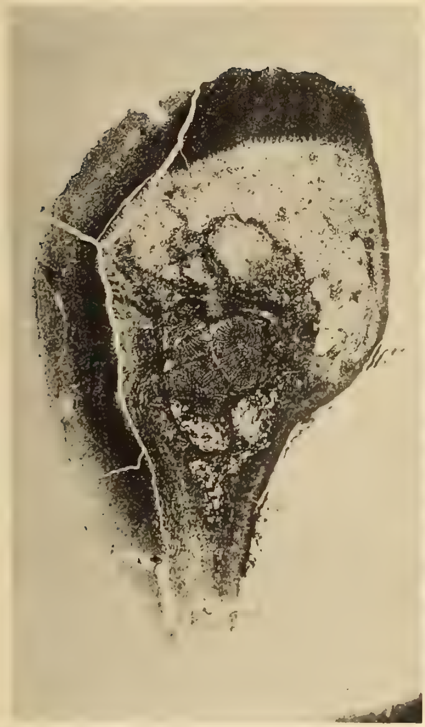

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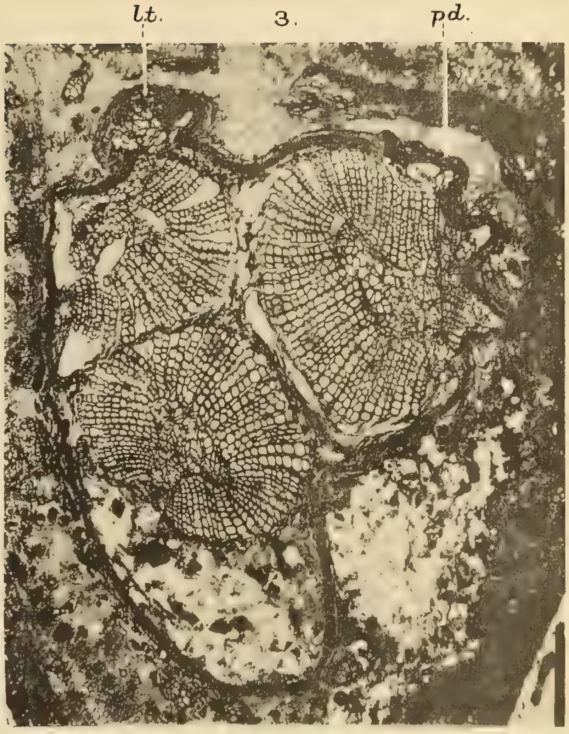

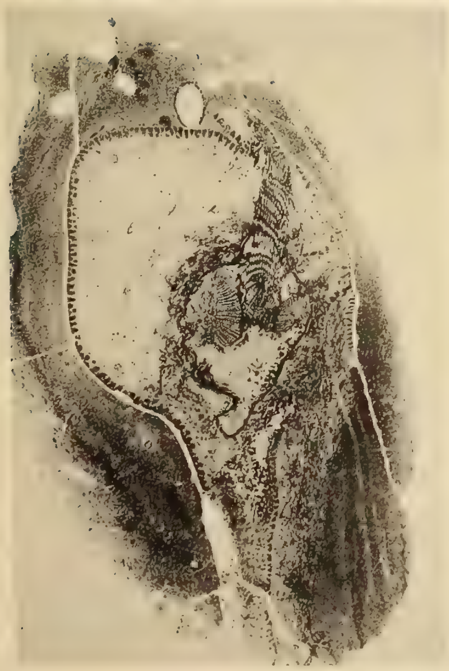

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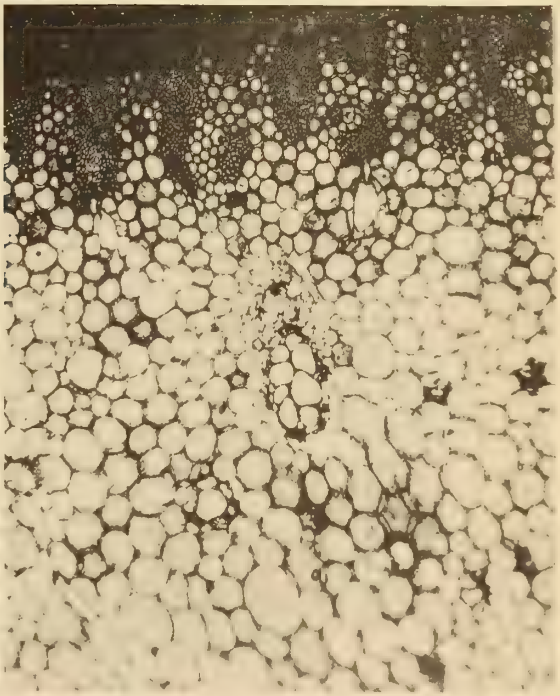

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SCOTT — MEDULLOSA PUSILLA.

The Origin and Destiny of Cholesterol in the Animal Organism. Part XI.—*The Cholesterol Content of Growing Chickens under Different Diets.*

By J. A. Gardner and P. E. Lander, Lindley Student of the University of London.

(Communicated by Dr. A. D. Waller, F.R.S. Received September 30,—Read November 20, 1913.)

(From the Physiological Laboratory of the University of London.)

In previous papers of this series evidence has been brought forward showing that cholesterol is a substance which is strictly conserved in the animal organism, and that waste of cholesterol can be made up from the food taken by the animal. Whether cholesterol can, to any extent, be synthesised in the organism from proteins, fats or carbohydrates, it is difficult to ascertain. It seemed likely that evidence on this point might be obtained by comparing the cholesterol content of eggs and newly hatched chickens, by studying the change of the cholesterol content with growth, and also by ascertaining whether chickens could be reared and would thrive on food deprived of its cholesterol and phytosterol. Parke,[*] as long ago as 1867, found that the quantity of matter which could be extracted by both ether and alcohol from the yolk of hen's eggs diminished during incubation, and he stated that cholesterol changes similarly. In 1908, L. B. Mendel and Leavenworth[†] published the results of some experiments to determine whether cholesterol is produced during the development of the eggs, and found that no increase took place, but that the cholesterol appeared to decrease, in part, in company with the rest of the lipoid substances. In Part IV of this series Ellis and Gardner[‡] concluded from a large number of analyses of eggs and chickens that in the differentiation of the ovum into the complex aggregates of cells constituting the chicken no formation of cholesterol takes place. Whether the cholesterol of the egg remained unchanged or whether some loss occurred could not be definitely decided. In this paper we give an account of our experiments on the growth of chickens under various diets, and comparisons of the cholesterol and cholesterol ester content of day-old chicks, and chicks at various stages of growth.

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[*] Parke, 'Hoppe-Seyler's Medizinisch-chemische Untersuchungen,' 1867, p. 211; for lecithin, *cf.* Mesernitzky, 'Biochemische Centralblatt,' 1907, vol. 6, p. 784.

[†] Mendel and Leavenworth, 'Amer. Journ. Physiol.,' 1908, vol. 21, pp. 82–84.

[‡] Ellis and Gardner, 'Roy. Soc. Proc.,' 1909, B, vol. 81, pp. 129–132.

Method of Estimation of the Cholesterol.

The weighed chickens were minced in a mincing machine and the minced mass was pounded up in a mortar with sand and sufficient plaster of Paris to cause the whole to set after a time to a dry mass, which was powdered and then extracted with ether in a Soxhlet's apparatus for about a fortnight. The ethereal solution of the extract was made up to a known volume and suitable aliquot proportions taken for analysis. The analyses were made by a modification of Windaus' digitonin method devised by Fraser and Gardner.*

General Plan of Experiments.

Fifty-four white Wyandotte day-old chicks of the same strain were obtained from a dealer and six of these were killed straight away and analysed with the following result.

The weight of the six chickens was 212 grm., and the extract was made up to 1 litre; 100 c.c. of this extract were then taken, and by direct precipitation gave 0·3407 grm. of the compound, corresponding to 0·0828 grm. of cholesterol, whilst 100 c.c. after saponification gave 0·49 grm. of the compound corresponding to 0·1189 grm. of cholesterol.

The remaining chickens were placed in four pens A, B, C, and D, as nearly as possible under the same conditions, 10 in A, 10 in B, 14 in C, and 14 in D. Each pen consisted of a foster mother, kept at a suitable uniform temperature by electric means, and was provided with a run with a sandy floor in which was placed a trough of water, and in which the animals were fed. Three diets were made use of.

(1) An ordinary commercial chicken food, of approximately the following composition:—

	Per cent.		Per cent.
Water	9·65	Fats	2·25
Proteins	22·50	Ash.....................	1·20
Carbohydrates......	64·40		

(2) The same chicken food as the above, which had previously been freed as far as possible from fats, cholesterol and phytosterol. In this food the percentage of protein was found to be about 19·9 per cent.

(3) The same diet as in (2), but with the addition of about 2 per cent. of cholesterol.

In the case of the first two meals given to the chickens, the diet (1) was replaced by ordinary hard-boiled egg, pounded up and moistened with warm water.

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* Fraser and Gardner, 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 560.

Diet (2) was replaced by the white of egg, and the yolk which had been extracted with ether.

Diet (3) was replaced by white of egg and extracted yolk to which about 2 per cent. of cholesterol had been added.

Pens A and B.—Ten chickens were placed in pen A, weighing altogether 336 grm. After one week the weight of these chicks was 512 grm.; 10 chickens were placed in B, weighing altogether 346 grm., and after one week their combined weight was 555 grm.

After one week two chickens were taken from pen A and three from pen B, their total weight being 260·1 grm. These were killed and analysed in the usual way, the total ether extract being then made up to 1 litre; 100 c.c. of this extract yielded by direct precipitation 0·228 grm of compound, corresponding to 0·0554 grm. of cholesterol, and after saponification 100 c.c. yielded 0·2908 grm. of the compound, corresponding to 0·0706 grm. of cholesterol.

After 14 *Days.*—The eight chickens in pen A weighed 559 grm., and the seven in pen B weighed 515·3 grm. From pen A three chickens weighing 205·5 grm. were taken, and from pen B two chickens weighing 150 grm., their total weight being 355·5 grm. These were killed and analysed with the following results:—The extract was made up to 1 litre; 100 c.c. of this gave by direct precipitation 0·271 grm. of compound, corresponding to 0·066 grm. of cholesterol, while after saponification 100 c.c. gave 0·365 grm. of compound, corresponding to 0·0887 grm. of cholesterol.

After 21 *Days.*—The five chickens in pen A now weighed 402·9 grm., and the five in B weighed 496·6 grm. From pen A two chickens were taken, weighing 199·1 grm., and from B two chickens weighing 152·5 grm., their total weight being 351·6 grm. These were killed and analysed in the usual manner, and the ethereal extract made up to 1 litre; 100 c.c. of this extract gave by direct precipitation 0·207 grm. of the compound, corresponding to 0·0505 grm. of cholesterol, whilst after saponification 100 c.c. gave 0·382 grm. of the compound, corresponding to 0·0829 grm. of cholesterol.

After One Month.—The three chickens in pen A now weighed 287·5 grm., and the three in pen B 361 grm. Two chickens were now taken from pen A, weighing 207·7 grm., and one chicken from pen B, weighing 103·5 grm., their total weight being 311·2 grm. These were killed and analysed in the usual manner, and ethereal extract was made up to 1 litre; 100 c.c. of this extract were taken, and by direct precipitation gave 0·2 grm. of the compound, corresponding to 0·0487 grm. of cholesterol, whilst 100 c.c. after saponification gave 0·2386 grm. of the compound, corresponding to 0·058 grm. of cholesterol.

In Pen C.—Fourteen chicks were in this pen, weighing altogether 766 grm. One of these died on the seventh day. Five chickens, including the dead one, were now taken and analysed in the usual way, their combined weight being 250·1 grm. The ethereal extract was made up to 1 litre, and the following results were obtained:—100 c.c. of the solution gave by direct precipitation 0·2767 grm. of the compound, corresponding to 0·0551 grm. of cholesterol, whilst after saponification 100 c.c. gave 0·3032 grm. of the compound, corresponding to 0·0737 grm. of cholesterol.

After 14 *Days.*—There were now nine chickens in pen C, weighing 657·5 grm.; four of these, weighing 295·6 grm., were killed and analysed, the ethereal extract being made up to 1 litre; 100 c.c. of this extract gave by direct precipitation 0·1646 grm. of compound, corresponding to 0·0400 grm. of cholesterol, while 100 c.c. after saponification gave 0·2341 grm. of compound, corresponding to 0·0569 grm. of cholesterol.

After 21 *Days.*—Pen C contained five chickens, weighing altogether 514·8 grm. None were killed this week, and

After 28 *days* the weight of the chickens was 614·5 grm. Three of these, weighing 357·7 grm., were killed and analysed, and the ethereal extract made up to 1 litre; 100 c.c. were taken, and by direct precipitation gave 0·216 grm. of compound, corresponding to 0·0525 grm. of cholesterol, and after saponification 100 c.c. gave 0·254 grm. of compound, corresponding to 0·0618 grm. of cholesterol.

Pen D.—Fourteen chickens, weighing 532·2 grm., were in this pen to start with.

After 7 *days* the weight of the chickens was 742·8 grm. Five chickens, weighing 249·8 grm., were killed and analysed with the following results:—The ethereal extract was made up to 1 litre; 100 c.c. of this extract gave by direct precipitation 0·272 grm. of compound, corresponding to 0·0661 grm. of cholesterol, while after saponification 100 c.c. of the extract gave 0·447 grm. of the compound, corresponding to 0·1097 grm. of cholesterol.

After 14 *days* the nine remaining chickens weighed 587·3 grm.; four of these, weighing 262 grm., were now killed and analysed with the following results:—The extract was made up to 1 litre, and 100 c.c. gave by direct precipitation 0·2432 grm. of compound, corresponding to 0·0511 grm. of cholesterol, while 100 c.c., after saponification, gave 0·4919 grm. of compound, corresponding to 0·1195 grm. of cholesterol.

After 21 *days* the five remaining chicks weighed 447·3 grm., and

After 28 *days* their weight was 583·5 grm.; three of these, weighing 352·5 grm., were now killed and analysed with the following results:—The extract was made up to 1 litre, 100 c.c. of this gave, by direct precipitation

0·4065 grm. of the compound, corresponding to 0·0988 grm. of cholesterol, whilst, after saponification, 100 c.c. gave 0·5518 grm. of the compound, corresponding to 0·134 grm. of cholesterol.

We do not think that the results obtained after the end of the second week are of much value for purposes of comparison, as the individual chickens showed such great variations in size and vigour. For instance, the odd chicks remaining in pens A, B, and C, at the end of the fifth week, weighed approximately 83, 159, and 115 grm., and the two chicks in pen D 258 grm.

Then, again, in pens A and B, where the animals were on the same diet and apparently under similar conditions, the growths showed considerable changes, as evidenced by the following table:—

	Pen A.	Pen B.
	100·0	100·0
First week	152·4	160·4
Second week	208·0	212·7
Third week	239·8	287·1
Fourth week	285·2	347·7

Whether this was due to the individuality of the animals, or whether it was due to differences in the amount of food taken, we are unable to say, as we gave excess of food, but had no means of measuring the amount left over.

An excess of food was given at definite times morning and evening, and the chickens were allowed to eat as much as they wished. It was impossible, however, to measure the amount of food eaten, owing to the habits of the birds, which trampled the food with the sand and excrement. It was impossible, therefore, either to estimate the unused food, or to examine the fæces, and it would have been impossible to have reared the chicks in a clean cage without sand, as they would not thrive under these conditions.

Furthermore, the laboratory conditions were not very suitable for healthy growth in prolonged experiments. The individual variations were, however, not so marked during the first two weeks, and we think that the results obtained with the various diets during this period are fairly comparable. We give, however, the later analyses for what they are worth.

In Table I we give the weights of the chickens at the various periods of growth, and the weights of cholesterol and cholesterol esters which they contain at those periods, calculated from the above data on the basis of 100 grm. of day-old chick.

Table I.

Age.	Diet of ordinary chicken food. (Animals from pens A and B.)				Diet of extracted chicken food. (Animals from pen C.)				Diet of extracted chicken food + cholesterol. (Chickens from pen D.)			
	Weight.	Total cholesterol.	Free cholesterol.	Ester cholesterol.	Weight.	Total cholesterol.	Free cholesterol.	Ester cholesterol.	Weight.	Total cholesterol.	Free cholesterol.	Ester cholesterol.
	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
1 day	100·0	0·561	0·391	0·170	100·0	0·561	0·391	0·170	100·0	0·561	0·391	0·120
7 days	156·5	0·476	0·334	0·142	148·6	0·438	0·328	0·111	139·6	0·613	0·369	0·244
14 ,, ...	210·0	0·528	0·391	0·137	198·5	0·383	0·276	0·107	171·7	0·784	0·387	0·396
21 ,, ...	263·8	0·624	0·380	0·244	279·6	—	—	—	235·4	—	—	—
28 ,, ...	319·9	0·590	0·496	0·095	333·8	0·579	0·491	0·087	306·8	1·169	0·861	0·308

Discussion of Results.—Dealing only with the results of the first two weeks, it will be seen from the table that, on the ordinary diet, the total cholesterol decreases by about 15 per cent. during the first week, and then increases again during the second week to within about 6 per cent. of the value in the day-old chick; the free cholesterol shows a similar decrease during the first week, but at the end of the second week has increased again to the original value. The ester cholesterol also decreases by about 17 per cent. during the first week, but the decrease is much less marked during the second.

In the case of animals fed on the extracted diet, there is a decrease in the total cholesterol during the two weeks, but the decrease during the first week is nearly double that in the second. This variation in the rate of decrease appears to be due to the ester cholesterol, which shows a marked decrease during the first week, but only a slight change during the second. The free cholesterol goes down fairly steadily.

In the case of the animals fed on extracted food + cholesterol, the total cholesterol increases during the two weeks, more markedly in the second than in the first. The cholesterol thus stored up is mainly in the form of esters; these show a marked increase during the two weeks, the increase in the second being double that in the first. The free cholesterol does not appear to be much affected; the figures are of much the same order as in the case of the animals on ordinary diet, the main difference being that the decrease noticed during the first week is less marked.

It will be noticed that the rate of growth of the animals in the earlier weeks on the cholesterol diet was slower than in the other cases, but we have no means of forming an opinion as to whether this is due to the diet, or to the amount of food eaten, or to the individuality of the animals. The day-old chicks, however, in this case were smaller than in the others.

What is the meaning of the high free cholesterol content in the fourth week of the experiment with extracted food we are unable to say, but we cannot attach much value to this particular result for the reasons already stated.

The cholesterol content of the chickens thus appears to depend on the sterol content of the diets, but there is nothing in the figures of the first two weeks to indicate that in the growing animal the organism can synthesise cholesterol. We have not carried out this investigation in as detailed a manner as was intended at the outset, as the animals did not prove very suitable for the investigation of problems of growth, partly owing to the habits of the birds, rendering it very difficult to control the amount of food taken, and to estimate the excrement, and partly owing to the difficulty of getting strictly comparable sets of birds. The laboratory conditions with strict control of diet and proper collection of excrement are not very suitable for keeping birds in health for a prolonged period. One of us is, however, extending his observations to the study of growing rats, which can be much more suitably reared and controlled, and hopes shortly to be able to publish an account of his experiments.

The results given in this paper, however, taken in conjunction with the fact that, in the differentiation of the ovum into the complex aggregate of cells constituting the chicken, no formation of cholesterol takes place, appear to support the view formerly expressed that cholesterol is not readily synthesised in the organism.

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for assistance in carrying out this work.

Contributions to the Biochemistry of Growth.[*]*—On the Lipoids of Transplantable Tumours of the Mouse and the Rat.*

By W. E. Bullock and W. Cramer.

(Communicated by Sir John Bradford, Sec. R.S. Received October 1,—Read November 20, 1913.)

(From the Chemical Laboratory of the Physiology Department, Edinburgh University, and the Imperial Cancer Research Fund, London.)

The following observations were carried out with the object of obtaining information concerning the presence of lipoid substances in rapidly growing cells. Our observations were made on transplantable tumours of mice and rats, which, as we have pointed out in previous papers of this series, are especially suitable for the study of the biochemical problems of growth. So far as we are aware, neither qualitative nor quantitative observations on the presence of lipoids in these tumours have as yet been made.

The analytical method employed was the same as the one used in our observations on normal and degenerating nerves.[†] The fact that by means of this method 99 to 101 per cent. of the total lipoids present in normal nervous tissue—which is free from ordinary non-phosphorised fat—can be accounted for is an indication of the accuracy of the method. Details of the method will be published in a separate paper. Here it may be sufficient to indicate the principle of the method, which consists in extracting the total lipoids by chloroform, and estimating in aliquot portions of the extracts: (1) the cholesterin and cholesterin esters by Windaus' method, using certain precautions which are essential for the accuracy of the results; (2) the phosphatides by a phosphorus estimation; (3) the cerebrosides by estimating the galactose liberated after prolonged acid hydrolysis; (4) the ordinary non-phosphorised fats (glycerides of fatty acids) by difference. In calculating the phosphatides and cerebrosides from the data obtained by this method, it is assumed that the average phosphorus percentage of the phosphatides and the galactose content of the cerebrosides present in the tumour cells do not differ essentially from the figures known for the phosphatides and cerebrosides of nervous tissue. It is, of course, possible that this assumption is not correct. In that case a recalculation of the figures given in this paper for the phosphatides and cerebrosides would be necessary. But since our arguments are based on a comparison between the figures obtained for different strains of tumours, such a correction would not materially affect our conclusions. In fact, in the case of the cerebrosides, for which the differences between different tumour-strains are—as will be seen—so great as to become qualitative, this possible source of error can be disregarded.

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[*] This research is in continuation of papers in 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 263; 1910, vol. 82, pp. 307 and 316; 1913, vol. 86, p. 302.

[†] Cramer, Feiss and Bullock, 'Proc. Physiol. Soc.,' 1913, p. li, 'Journ. Physiol.,' vol. 46.

The observations refer to a rapidly growing rat sarcoma, and to two different strains of a mammary carcinoma of the mouse, of which one always grows rapidly (63/73B), while the other always grows slowly (72/21A and 72/22A). Since the rapidly growing mouse carcinoma showed marked necrosis, the macroscopically necrotic parts were separated from the less necrotic portion of the tumour and analysed separately. These two parts are described in the following table as "necrotic part" and "healthy part" respectively; but it must be understood that even in the macroscopically healthy parts there was still a good deal of necrotic tissue. The rat sarcoma showed little necrosis.

The material subjected to analysis was obtained in the case of each strain from several tumours belonging to the same subtransplantation. The figures given in the following table represent therefore the composition of the lipoids of a number of sister tumours at one particular period of their growth, for the three tumour strains; they do not merely indicate the composition of one individual tumour.

Analysis of Lipoids of Transplantable Tumours.

	Carcinoma of mouse. Slow growth. No necrosis.		Carcinoma of mouse. Rapid growth.				Sarcoma of rat. Rapid growth. No necrosis.	
			Healthy part.		Necrotic part.			
	Dry tissue.	Total Lipoids.	Dry tissue.	Total Lipoids.	Dry tissue.	Total Lipoids.	Dry tissue.	Total Lipoids.
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Total lipoids ……	13·3	—	13·1	—	41·1	—	18·5	—
Cholesterin………	1·1	8·8	Trace	0	Trace	0	1·4	7·8
Cholesterin esters	0	0	0	0	0	0	0	0
Phosphatides……	0·88	6·6	1·3	9·7	2·54	6·2	7·2	38·8
Cerebrosides ……	0	0	0	0	Trace ?	0	1·9	10·1
Ordinary fats……	11·32	84·6	11·8	90·3	38·56	93·8	8·0	43·3

A comparison of the slowly growing mouse tumour with the healthy parts of the rapidly growing mouse tumour shows that (1) the percentage of total lipoids is practically the same; (2) cholesterin forms 1 per cent. of the total solids of the slowly growing tumour, while it is present only in traces in the rapidly growing tumour; (3) phosphatides form only a small proportion of both tumours, though they are more abundant in the rapidly growing tumour; (4) both tumours are devoid of cerebrosides and cholesterin esters; and (5), that the bulk of the total lipoids of these two tumours consists of ordinary fats.

The change in composition brought about by necrosis is shown by a comparison of the figures for the healthy and necrotic parts of the rapidly growing tumour. The most conspicuous feature of the change is the increase in total lipoids, which are trebled in amount, the increase consisting to a great extent of ordinary fat, and to a less extent of phosphatides. A trace of a reducing substance is liberated by acid hydrolysis from the lipoids of the necrotic parts, but whether this can be taken as an indication of the formation of small amounts of cerebrosides during necrosis must for the present remain doubtful.

It is of particular interest to note that different strains of mammary mouse carcinoma may show such wide variations in chemical composition, as is shown here between the relative proportion of different lipoids, and more especially of cholesterin. It has been pointed out in numerous papers from the Imperial Cancer Research Fund that marked biological differences exist between the cells of different tumour-strains, even although these cells may originally be derived from the same tissue (mamma). It is interesting to note that there exist also chemical differences between cells derived from the same tissue.

It is, of course, tempting to refer these differences to differences in the rate of growth. But such a conclusion can only be based on a systematic series of analyses carried out with different strains, and with the same strain at different periods of its growth. Such observations are now being carried out by one of us (W. E. B.).

When we compare the lipoids of tumours derived from different tissues—namely, a rat sarcoma with a mouse carcinoma—we find very marked differences, both quantitatively and qualitatively. The amount of total lipoids present in the sarcoma does not differ very markedly from that present in the carcinomata, especially when we take into account that the analyses refer to two different species. But there is a complete change in the proportion in which the various lipoids are present. While in the two carcinomata the bulk of the total lipoids is made up of ordinary non-phosphorised fats, and phosphorised fats are present only in relatively small amounts (from 7 to 10 per cent. of total lipoids), the phosphorised fats of the sarcoma represent almost 40 per cent. of the total lipoids and equal in amount the ordinary non-phosphorised fats. Cholesterin is present in quantities similar to those found in the slowly growing carcinoma, while cholesterin esters are absent both in the sarcoma and in the carcinomata. Perhaps the most interesting feature is the presence in the sarcoma cells of definite amounts of cerebrosides (2 per cent. of dry tissue). These lipoid substances are completely absent from the two mammary carcinomata which we have analysed, and the question arises, whether this qualitative chemical difference is typical for carcinoma and sarcoma, or whether it is exhibited merely by individual tumour-strains. This problem, again, must remain a subject for further inquiry.

Summary.

The cells of different strains of transplantable tumours show quantitative and qualitative differences in the various groups of lipoid substances present. Such differences exist not only between tumour-cells derived from different tissues—namely, a sarcoma and a carcinoma, but also, although not to the same extent, between two different strains of tumour-cells derived from the same tissue—namely, a slowly growing and a rapidly growing mammary carcinoma.

Necrotic tumour-cells show a great increase in the amount of total lipoids present, the increase being due mainly to an increase in ordinary non-phosphorised fats.

The expenses of this research were defrayed by grants from the Moray Fund of the University of Edinburgh.

Studies in Heredity. II.—*Further Experiments in Crossing British Species of Sea-urchins.**

By E. W. MacBride, F.R.S.

(Received October 27,—Read November 6, 1913.)

Two years ago I read a paper before the Society in which I gave the results which I had obtained by crossing the two species of sea-urchin *Echinus esculentus* and *Echinocardium cordatum.* The first of these is a regular urchin, the second an enocyclic urchin belonging to the order Spatangoidea. The results recorded in that paper were briefly these :—The egg of *Echinocardium* fertilised by the sperm of *Echinus* gives rise to a hybrid which exhibits a mixture of maternal and paternal characters, but only a small proportion of the hybridised eggs develop. The egg of *Echinus* fertilised by the sperm of *Echinocardium* develops a fertilisation membrane, but then breaks up into a heap of globules by the process known as cytolysis, and dies.

Since writing that paper my experiments were repeated at Plymouth by Fuchs,† now my colleague at the Imperial College, and he obtained different results. According to him the eggs of *Echinus* fertilised by the sperm of *Echinocardium* gave rise to hybrid larvæ which were maternal in character, whilst the eggs of *Echinocardium* fertilised by the sperm of *Echinus* gave rise to hybrids of two kinds, some purely maternal in type and some resembling those which I had obtained.

As I suspected that some of the divergences between the results of Fuchs and myself might be due to the accidental infection of the sea-water used with the sperm of the species of which the eggs were taken, a circumstance which would produce larvæ of purely maternal character, I resolved to repeat my experiments, and accordingly this summer I proceeded to Millport, where my first experiments were performed. I have to thank Mr. Elmhirst, Superintendent of the Marine Biological Station at Millport, for the way in which he aided my efforts. To his kindness I owe the successful issue of my experiments.

The results which I obtained during this last summer prove that my suspicion as to the cause of the divergence between Fuchs's results and my own was entirely unfounded, for this year I obtained larvæ by fertilising the eggs of *Echinus* with the sperm of *Echinocardium*, which were purely maternal in character. I may add that, in addition to repeating the experiments which I had performed two years before, I also crossed the species *Echinus miliaris* with *Echinocardium cordatum*, but this species gave practically the same results as *Echinus esculentus*, and, except where otherwise stated, the remarks in this paper will apply to the cross between *Echinus esculentus* and *Echinocardium cordatum.*

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* "Studies in Heredity. I.—The Effects of Crossing the Sea-urchins *Echinus esculentus* and *Echinocardium cordatum,*" 'Roy. Soc. Proc.,' 1911, B, vol. 84.

† "The Inheritance of the Aboral Process of the Echinocardium Pluteus," 'Arch. Entw.-Mech.,' 1912, vol. 35.

Every possible precaution was adopted to prevent sperm infection The outsides of all the urchins used were washed in a copious stream of fresh water before they were opened, in order to destroy any adhering sperm; all the instruments employed were likewise washed in fresh water, and the fertilisation of the eggs was effected in sea-water, which had been thoroughly sterilised by previous heating to 80° C.

One fact which forced itself prominently on one's notice this year was the individual idiosyncrasy of urchins with regard to the capacity for being crossed. Sometimes two urchins when crossed, although to all appearance ripe, would not yield a single hybrid larva—yet the eggs developed rapidly when fertilised with their own sperm. Another time, females apparently only half ripe yielded eggs which when fertilised with the foreign sperm produced a certain proportion of hybrid larvæ.

In general it may be said that the eggs of *Echinocardium cordatum* in 1913 were much more resistant to the action of the sperm of *Echinus esculentus* than they were in 1911. In some cases these eggs refused to develop at all, but broke up by the process of cytolysis into a multitude of spherules, just as I had described the eggs of *Echinus esculentus* doing when fertilised with the sperm of *Echinocardium cordatum* (fig. 1). Indeed, in all cases this may be said to have been the case of most, but in many cases a certain proportion of hybrid larvæ were produced which showed in every detail the features which I had described in the hybrids obtained in 1911, and I shall not therefore

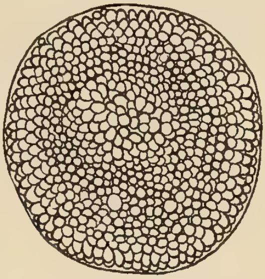

Fig. 1.—Egg of *Echinocardium cordatum*, fertilised with the sperm of *Echinus esculentus* breaking up into globules.

repeat them here. Unfortunately I could not keep any of them alive for a longer period than five days, and therefore I did not obtain one of the features (viz., the inbending of the aboral ends of the body rods) which only appeared in hybrids which in 1911 had lived longer than five days.

Few as were the hybrids obtained by fertilising the eggs of *Echinocardium* with the sperm of *Echinus,* still fewer were the hybrids resulting from the fertilisation of the eggs of *Echinus* with the sperm of *Echinocardium.* In the vast majority of cases the eggs broke up with the formation of globules as I described in 1911. In the case of one urchin I obtained larvæ which were purely maternal in character, *i.e.* which resembled exactly the normal larvæ of *Echinus esculentus.* This result therefore tallied with that which Fuchs had obtained in 1912, indeed the larvæ which I obtained lived longer and developed further than those described by Fuchs. In all other cases very peculiar hybrids were produced. The egg segmented so as to form a regular blastula, but when the primary mesenchyme cells were budded off into the blastocœle (fig. 2) the divergence between the hybrid and normal larvæ became clearly marked. In the normal blastula only about fifty primary mesenchyme cells are produced, all of about the same size, and these are arranged in a ring, but in the hybrid not only are the mesenchyme cells of many different sizes but some of them show the beginnings of the process of cytolysis. The conclusion is irresistibly suggested to the mind that this over-production of mesenchyme is really a manifestation of the

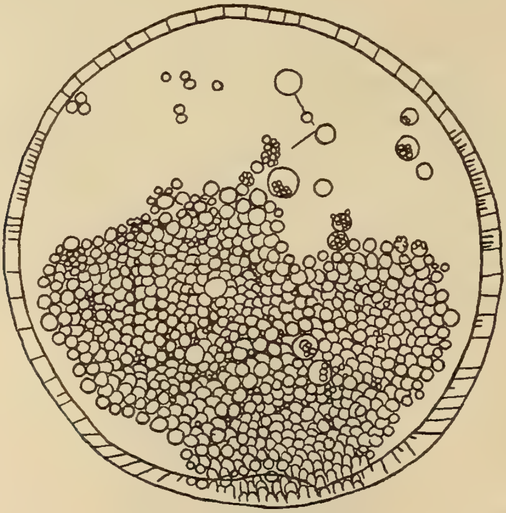

Fig. 2.—Blastula produced by fertilising the egg of *Echinus esculentus* with the sperm of *Echinocardium cordatum.*

tendency to cytolysis, which inhibits altogether the development of most eggs of Echinus when treated with the sperm of *Echinocardium.*

The next day the hybrid blastula becomes a gastrula by the appearance of the invagination which gives rise to the archenteron. This invagination is of very small size compared to the corresponding invagination in a normal gastrula—it is, so to speak, pushed to one side by the abnormal multitude of mesenchyme cells (fig. 3). On the third or fourth day the larva reaches the stage when the normal larva is denominated a "prism-larva"—the stage, that is to say, when the archenteron has become differentiated into œsophagus, stomach and intestine, and when the mouth and stomodæum are making their appearance as a new invagination. The hybrid, however, has retained the spherical form of the egg; it is filled with a great mass of mesenchyme, and the small and feebly developed alimentary canal lies pushed to one side (fig. 4).

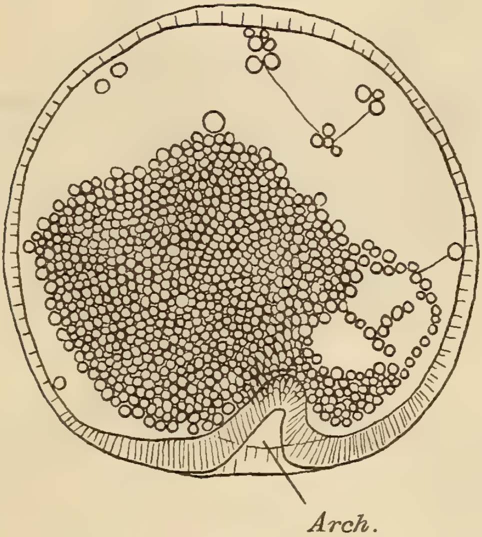

FIG. 3.—Gastrula produced by fertilising the egg of *Echinus esculentus* with the sperm of *Echinocardium cordatum.* *Arch*, archenteric invagination.

A few of these hybrids lived to the age of five or six days, and, whilst the form remained mainly spherical, two short feeble rudiments of the postoral arms were produced.

Loeb,* in describing experiments with the eggs of *Asterias,* makes the statement that these eggs require to lie in sea-water for some time in order to ripen: and that after they have lain for a day or so in sea-water without being fertilised they die and undergo granular degeneration. It occurred to me that a critic might possibly advance the theory that the eggs which I believed to have undergone cytolysis, in consequence of the entry of foreign sperm, really died in the normal course of affairs through the inability of the foreign sperm to enter them and the consequent absence of fertilisation. In the case of *Echinus* eggs the reply might be made that these even when undergoing cytolysis show clearly and unmistakably the fertilisation membrane, which may be accepted as evidence that a spermatozoon has entered the egg, but the egg of *Echinocardium* (fig. 1) does not show the fertilisation membrane clearly; if present it lies close to the surface of the egg. In order therefore to place the matter beyond doubt the following experiment was made:—

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* 'Die Chemische Entwicklung des Tierischen Eies,' Jena, 1910.

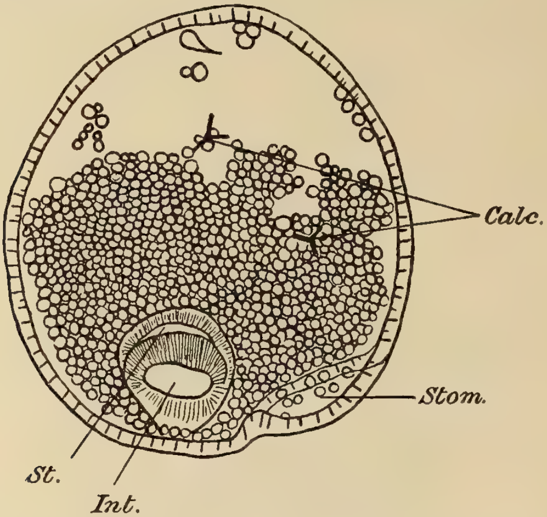

Fig. 4.—Stage equivalent to the "prism-larva" in a hybrid produced by fertilising the egg of *Echinus esculentus* with the sperm of *Echinocardium cordatum*. *Calc.*, incipient calcifications, the rudiments of the larval skeleton; *Int.*, intestine; *St.*, stomach; *Stom.*, stomodæum (ectodermal œsophagus).

Eggs were shaken out from a ripe *Echinocardium* into sterilised sea-water. The culture thus obtained was divided into two portions: one portion (A) was treated with the sperm of *Echinus esculentus*, and one portion (B) was left unfertilised. On the following morning, in (A) all the eggs were dead and had undergone cytolysis, but all the eggs in (B) were as clear and transparent as when they had left the parent. (B) was now divided in three portions, which we shall designate (*a*), (*b*), and (*c*); (*a*) was fertilised with *Echinocardium* sperm, (*b*) was fertilised with *Echinus* sperm, and (*c*) was left unfertilised. On the following morning, (*a*) had given rise to numerous larvæ, showing that the eggs of which it was composed were still perfectly capable of development. The eggs of (*b*), however, had neither developed nor had they undergone cytolysis. They had remained clear, transparent, and unaltered, although they were surrounded by forests of spermatozoa which had failed to enter them. The eggs of the portion (*c*) had likewise remained clear and unaltered.

It is therefore proved that the entry of a foreign spermatozoon into an egg may cause its death by producing cytolysis, and the enormous production of mesenchyme which takes place in those few hybrid eggs which do develop is in all probability a phenomenon of the same kind as cytolysis, which is after all only a premature and exaggerated fragmentation of the cytoplasm. But an egg can become totally unreceptive to foreign spermatozoa, whilst it is still capable of receiving the spermatozoa belonging to its own species and of undergoing normal development, and thus two causes of sterility when distinct species are crossed are unmasked, viz., either (*a*) the egg refuses to receive the foreign spermatozoon at all or (*b*) it receives it and undergoes cytolysis in consequence.

The Optimum Temperature of Salicin Hydrolysis by Enzyme Action is Independent of the Concentrations of Substrate and Enzyme.

By Arthur Compton, B.A., M.B., R.U.I., Imperial Cancer Research Fund.

(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S. Received October 14,—Read December 4, 1913.)

The object of the present investigation is to ascertain the influence, if any, on the optimum temperature—temperature of greatest activity—of an enzyme, of the concentration, on the one hand, of the substrate, and, on the other, of the enzyme. The investigation, involving two variables, presents three cases for consideration, according as the concentration of the substrate and the concentration of the enzyme are varied separately or together. An account is given of the results obtained with the enzyme or enzymic function, present in sweet-almond emulsin, which hydrolyses the glucoside salicin with the production of equimolecular quantities of glucose and saligenin. A commercial specimen of Merck's emulsin was used, while the purity of the salicin employed was ascertained by determining its melting point (200·5°) and its optical activity ($[\alpha]_D^{21} = -62{\cdot}7°$).

The successive stages in the inquiry may be briefly summarised as follows: (1) a preliminary determination of the activity of the specimen under certain chosen conditions as regards the concentration of the substrate, the temperature, and the duration of the experiment; (2) a preliminary determination of the optimum temperature with the quantity of enzyme found capable of producing 50 per cent. hydrolysis of the substrate under the above conditions; (3) a determination of the activity curves of the enzyme at the temperature thus found, in an action of the same duration for five concentrations of the substrate M/5, M/10, M/15, M/30, and M/50; (4) a determination of the optimum temperature of the enzyme for each of the five concentrations of the substrate in presence of a constant enzyme concentration; (5) a determination of the optimum temperature of the enzyme for each of the five concentrations of the substrate with quantities of enzyme indicated by the activity curves as capable of producing 70 per cent. hydrolysis of the substrate in the given time: (6) a determination of the optimum temperature of the enzyme for a constant concentration of the substrate in presence of different enzyme concentrations.

The preliminary determination of the activity of the enzyme was carried out in a M/5 dilution of the substrate during a period of 15 hours at 40°. The practical details were as follows: 286 mgrm. of salicin and varying quantities of the enzyme dissolved in 5 cm.^{3} of water, specially purified by redistillation under diminished pressure, were introduced into each of a series of seven clean Jena glass test-tubes. The tubes were incubated for 15 hours in a water thermostat at 40°, after which the enzyme action was stopped by rapidly cooling the tubes and then adding to each a drop of concentrated solution of ammonium hydroxide. The proportion of glucoside hydrolysed in each tube was estimated by the increase of reducing power, measured by the method of Bertrand.* The numbers obtained are set out in Table I.

Table I.

Quantity of enzyme.	Salicin hydrolysed.
mgrm.	per cent.
0·6	34·8
1·5	67·2
3·0	86·8
4·5	91·2
6·0	91·7
7·0	96·1
9·0	97·0

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* 'Bull. Soc. Chim.' [3], 1906, vol. 35, p. 1285.

If the percentages of salicin hydrolysed be plotted as ordinates and the quantities of enzyme as abscissæ these numbers give the activity curve shown in fig. 1.

The preliminary determination of the optimum temperature of the enzyme, the second stage of the inquiry, was carried out as follows: A solution of the enzyme was prepared containing 10 times the quantity of enzyme necessary to produce the percentage of hydrolysis decided upon—in this case 0·9 mgrm. for a 50 per cent. hydrolysis as shown by fig. 1—dissolved in 10 cm.^{3} of redistilled water. After half to one hour of contact at the ordinary temperature the solution was introduced in portions of 1 cm.^{3} into each of a series of eight or nine test-tubes already containing 286 mgrm. of salicin and 4 cm.^{3} of water. The tubes were then plunged into water-baths kept at known temperatures, and after 15 hours the action was stopped and the proportion of glucoside hydrolysed determined as before. The numbers obtained are set forth in Table II.

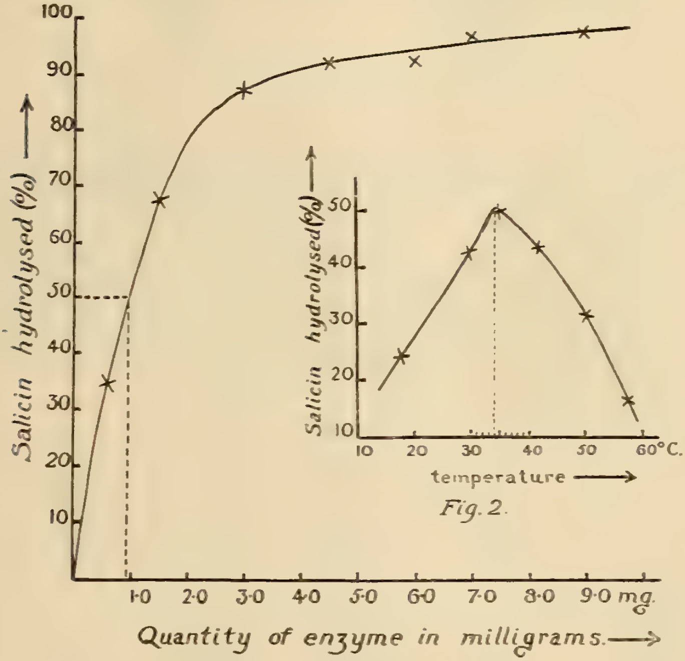

Fig. 2.

Fig. 1.

By plotting the percentage of salicin hydrolysed against the mean temperature of the experiment these numbers give the curve indicated above in fig. 2. The optimum temperature under the foregoing conditions is thus found to be +34°.

Table II.

Temperatures at the beginning and end of each experiment.	Salicin hydrolysed.
°	per cent.
17·8–17·6	24·2
29·3–29·5	42·8
34·7	50·0
41·8–41·7	43·0
50·2–50·3	31·4
57·5–57·6	16·4

Next the activity of the enzyme was determined in the vicinity of +34° for a 15 hours' action with each of the following concentrations of the substrate: M/5, M/10, M/15, M/30, and M/50, the effect of which on the optimum temperature of the enzyme it was ultimately intended to study. The temperature actually employed was 33·6°–33·8°; and the experimental details were the same, except the dilutions, as already described for the preliminary determination. The numbers obtained are given in Table III.

Table III.

Quantity of enzyme.	Salicin hydrolysed per cent. for the following concentrations:—				
	M/5.	M/10.	M/15.	M/30.	M/50.
mgrm.					
0·5	—	31·4	35·8	28·2	—
1·0	55·1	57·5	58·5	49·5	36·9
2·0	79·4	84·7	88·7	77·0	—
3·0	88·3	93·9	97·5	90·0	77·2
5·0	94·2	97·5	99·6	99·0	93·9
7·0	94·9	96·7	99·2	100·6	100·0
10·0	95·7	100·2	99·6	—	100·5
12·5	95·7	—	100·6	100·6	100·0

These numbers give, on plotting the percentage of salicin hydrolysed against the quantity of enzyme in play, the activity curves shown in fig. 3.

The influence of the substrate concentration on the optimum temperature of the ferment, the fourth stage of the inquiry, may now be considered. This is the case of determining the optimum temperature in a series of experiments in which the concentration of the enzyme is kept constant while that of the substrate varies. The concentration of the enzyme chosen, in accordance with fig. 3, was 0·4 mgrm. in 5 cm.^{3}, *i.e.* 8×10^{-5} grm. per cm.^{3} of the reaction mixture. Five different solutions of the enzyme were prepared

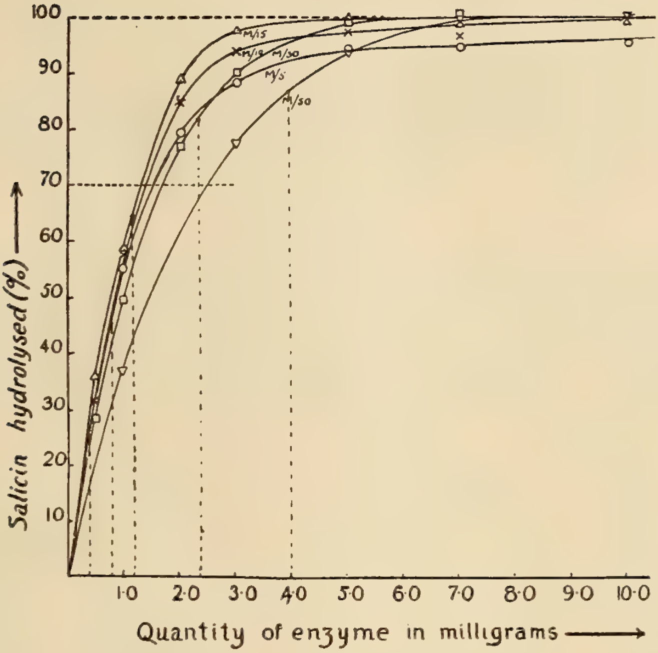

Fig. 3.

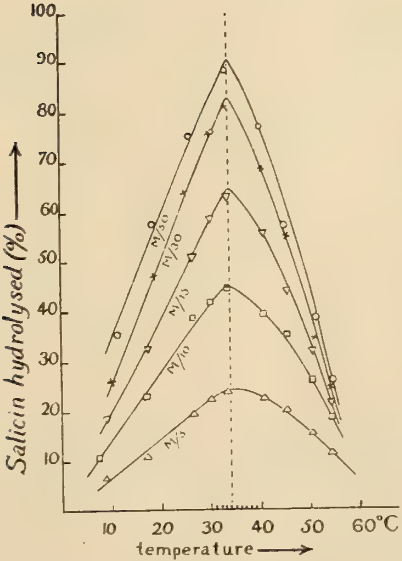

Fig. 4.–{Substrate concn. M/5 to M/50
Enzyme concn. 8× 10^{-5} gr. per cm^3.

containing 4, 8, 12, 24, and 40 mgrm. dissolved in 10 cm.^{2} of water, which, after standing for half to one hour, were introduced in portions of 1 cm.^{3} into five series of test-tubes containing 286 mgrm. of salicin and 4, 9, 14, 29, and 49 cm.^{3} respectively of water. After 15 hours' incubation in baths at known temperatures the action was stopped and the quantity of salicin hydrolysed in each tube estimated as before. The numbers obtained are given in Table IV.

Table IV.

Temperatures at the beginning and end of each experiment.	Salicin hydrolysed per cent. with the following substrate concentrations :—				
	M/5.	M/10.	M/15.	M/30.	M/50.
°					
7·3– 7·7	—	10·8			
8·3– 9·0	6·6				
9·6– 8·5	—	—	18·3		
9·4–10·8	—	—	—	26·0	
12·0–10·9	—	—	—	—	35·5
17·2–17·3	—	23·0			
17·4–17·1	10·8				
17·5–17·6	—	—	32·5		
18·6–18·5	—	—	—	—	57·5
18·9	—	—	—	47·0	
25·2–25·0	—	—	—	63·7	
26·4–25·7	—	—	—	—	75·0
26·5	19·2	38·6	50·8		
30·0	22·3	41·9	58·5	75·3	
30·5	—	—	—	—	75·8
33·5	23·6	44·6	62·9	80·7	88·3
40·5	—	—	—	68·3	76·7
40·7–40·6	—	39·4			
40·8–40·7	22·4	—	55·2		
45·0–45·2	—	—	—	—	56·9
45·5–45·3	19·7	35·0	43·7		
45·5	—	—	—	54·7	
50·3–50·5	15·1	—	31·9		
50·6–50·5	—	25·8	—		
51·1–50·8	—	—	—	34·3	
51·5–51·2	—	—	—	—	38·3
54·3–54·0	11·2				
54·2–54·3	—	18·4	21·4	24·3	
54·6–55·0	—	—	—	—	25·8

These numbers give graphically the curves shown in fig. 4 (p. 249).

On examination the above curves indicate, although with very different degrees of precision, maxima in the same region of temperature. In so far as the curves are comparable with one another, they produce the general impression that the optimum temperature of the enzyme is constant, and consequently independent of the concentration of the substrate. But to answer the question more definitely curves of a uniform type, easily comparable among each other, are required. This can only be achieved by varying the concentration of the enzyme at the same time as that of the substrate, for, *ceteris paribus*, the extent of an enzyme action is, as shown by the activity curves of figs. 1 and 3, determined by the proportion of enzyme to substrate present in the reaction mixture. The case which constitutes the fifth stage of the inquiry will now be considered.

Table V.

Temperatures at the beginning and end of each experiment.	Salicin hydrolysed per cent. with the following molecular concentrations of the substrate and grm. of the enzyme per cm.^{3}.					
	M/5. 31×10^{-5}.	M/10. $13·5 \times 10^{-5}$.	M/15. $8·7 \times 10^{-5}$.	M/30. $5·7 \times 10^{-5}$.	M/50. 5×10^{-5}.	
°						
8·0	—	—	17·2			
9·0– 8·3	—	17·3				
9·5– 9·0	—	—	—	18·8		
17·8–17·5	35·0					
18·1–18·2	—	34·2				
18·2–18·4	—	—	—	—	39·7	
18·4	—	—	—	—	—	41·9
18·7–18·5	—	—	—	40·3		
18·8–18·7	—	—	36·3			
23·1–22·5	46·1					
24·9–25·1	—	—	—	—	50·5	
26·1–26·2	—	—	53·3			
26·2–26·4	—	—	—	50·3	—	61·1
27·0	—	56·9				
29·7	62·2					
30·0–30·5	—	—	—	65·7		
30·5	—	—	—	—	68·6	69·4
31·8–31·6	—	—	63·7			
33·5–33·8	—	69·4	65·8			
33·8	—	—	—	70·0	—	70·0
34·7–34·8	—	—	—	—	71·7	
35·6–35·7	—	—	65·8			
36·0	—	69·4				
36·6	—	—	—	66·3		
39·4–39·9	—	—	—	—	59·7	
41·8–42·0	60·5	58·3				
42·5–43·0	—	—	—	56·3		
43·0	—	—	—	—	—	53·0
43·6–43·5	—	—	51·2			
44·0–44·4	—	—	—	—	47·0	
47·8–48·0	—	43·3				
48·0–48·5	—	—	—	37·7		
48·5	—	—	37·7	—	—	36·4
49·0–49·5	—	—	—	—	34·1	
50·2	44·6					
51·0	—	35·6				
51·7–51·5	—	—	30·7			
53·5	—	—	—	22·7		
53·5-53·8	—	—	—	—	22·5	22·4
54·6	33·6					
57·5–57·4	23·7					

That the curves might be vertical enough to give sharply defined maximum points, it was decided to aim at obtaining about 70 per cent. hydrolysis of the substrate at the optimum temperature. A cursory examination of fig. 3, which was constructed at approximately +34°, shows that to obtain such curves—assuming for the moment, what fig. 4 already indicates the probability of, that the optimum temperature is independent of the concentration of the substrate, and situated at about + 34°—the quantities of enzyme required, in actions of 15 hours' duration, are 1·55, 1·35, 1·30, 1·70 and 2·50 mgrm. respectively for the concentrations M/5, M/10, M/15, M/30 and M/50 of the substrate. Working with these quantities the experimental data obtained are set forth in Table V.

By plotting as before the percentage of salicin hydrolysed against the temperature of the experiment the foregoing numbers give the curves represented in figs. 5, 6, 7, 8 and 9.

On careful examination the curves below all show that the activity of the enzyme is greatest between +33·5° and +34·5°; in other words, that the optimum temperature is about +34°, and is constant, notwithstanding the wide variations in the dilution of the substrate and the accompanying variations in the dilution of the enzyme.

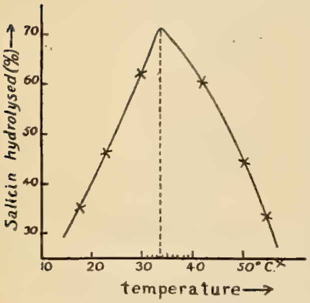

Fig. 5.–{Substrate conc^n M/5. Enzyme conc^n 31×10^{-5} gr. per cm^3

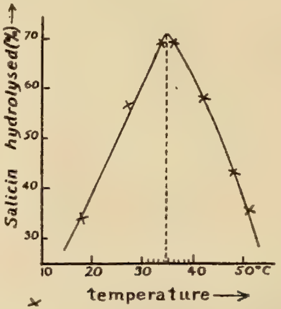

Fig. 6.–{Substrate conc^n M/10. Enzyme conc^n $13{\cdot}5\times10^{-5}$ gr. per cm^3

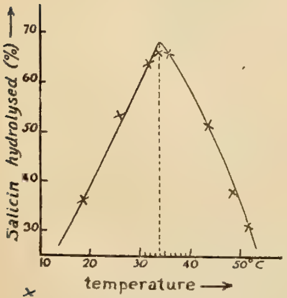

Fig. 7.–{Substrate conc^n M/15 Enzyme conc^n $8{\cdot}7\times10^{-5}$ gr. per cm^3

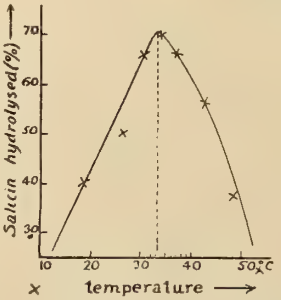

Fig. 8.–{Substrate conc^n M/30 Enzyme conc^n $5{\cdot}7\times10^{-5}$ gr. per cm^3

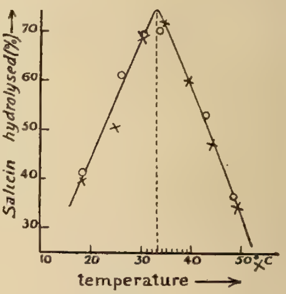

Fig. 9.–{Substrate conc^n M/50. Enzyme conc^n 5×10^{-5} gr. per cm^3

Table VI.

Temperatures at the beginning and end of each experiment.	Salicin hydrolysed per cent. with the following enzyme concentrations per cm.^{3}.					
	1·8 × 10^{-5}.	3·3 × 10^{-5}.	6·8 × 10^{-5}.	11·7 × 10^{-5}.	15 × 10^{-5}.	18 × 10^{-5}.
°						
8·5– 8·3	—	—	—	—	43·7	
9·5– 8·5	—	—	—	—	—	48·7
10·0–10·5	—	16·0				
17·5	—	—	—	—	70·0	
17·8–17·9	—	24·7				
18·2–18·0	—	—	—	—	—	78·7
18·7–18·6	—	—	—	63·0		
19·0	18·3					
19·5–19·0	—	—	47·7			
20·3–20·1	—	—	—	—	—	84·0
21·5–21·1	—	—	51·6			
22·5	—	—	—	—	—	92·0
24·4	—	—	—	78·0		
24·8–24·9	—	—	61·7			
25·5–25·6	22·5					
25·8–25·6	—	38·0				
26·2	—	—	—	—	90·0	
30·0	—	—	—	—	95·3	
30·2	25·2	46·8				
30·4–31·6	—	—	—	91·0		
31·1	—	—	—	—	—	99·0
31·6–31·7	—	—	76·0			
32·9–33·1	—	—	—	94·7		
33·5	—	46·8	—	—	98·0	
34·0	25·2					
37·6–37·8	—	—	74·2			
40·0–39·9	—	—	—	—	—	99·7
40·2	23·0					
40·5	—	43·3	—	85·0		
40·7	—	—	—	—	99·0	
41·8–42·0	—	—	65·3			
45·0–45·2	—	35·8	—	73·3	84·0	
45·0–45·4	18·8					
49·1–49·0	13·5					
50·5–50·4	—	—	—	—	61·3	69·0
51·1–51·0	—	—	—	47·7		
52·2–51·6	—	—	—	—	—	60·7
53·6–53·5	—	—	26·8			
54·0	—	14·6	—	—	42·7	
54·3	—	—	—	35·0		
55·0	—	—	—	—	—	44·3
56·0	6·5					
57·0	—	—	18·0			

Turning now to the last stage of the inquiry, the case of the substrate concentration remaining constant while that of the enzyme changes, it constitutes the study, properly speaking, of the influence of the enzyme concentration on the optimum temperature. Although rendered unnecessary by what precedes, the study is given in order to complete the present investigation. For this a M/30 dilution of the substrate was chosen and the optimum temperature determined in actions of 15 hours' duration with quantities of the enzymic specimen giving concentrations varying between $1{\cdot}8 \times 10^{-5}$ and 18×10^{-5} grm. per cm.^{3}. The numbers obtained are given in Table VI. The results are recorded graphically in fig. 10.

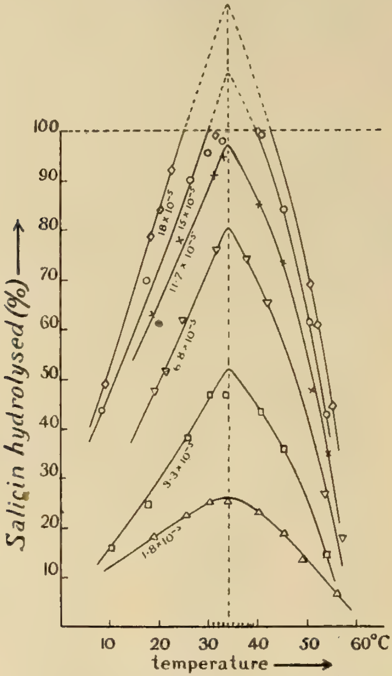

Fig. 10.—{ Substrate concn. M/30. Enzyme concn. $1{\cdot}8 \times 10^{-5}$ to 18×10^{-5} gr. per cm.^{3}

The curves of fig. 10, as well as the M/30 curve of fig. 4 and that of fig. 8, show that the optimum temperature of the enzyme is the same in each, and, consequently, independent of the concentration of the enzyme. This holds true, as shown by two of the curves in fig. 10, even when the proportion of enzyme to substrate is more than sufficient to produce complete hydrolysis of the substrate at the optimum point. Here the optimum point is imaginary, and corresponds to the intersection of the curves representing respectively the activation and the destruction of the enzyme by heat.

Briefly, then, the outcome of the inquiry is, for an action of known duration, the optimum temperature of the enzyme investigated is independent alike of the concentration of the substrate and of the concentration of the enzyme. Whether the statement be true of enzymes in general—as theoretical considerations would lead one to expect—I propose to answer by fresh experiments on other types of enzymes.

The Resonance of the Tissues as a Factor in the Transmission of the Pulse and in Blood Pressure.

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(Received June 24,—Read June 26, 1913.)

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Systolic blood pressure in man is measured by the pressure indicated on a manometer scale at the point of disappearance and reappearance of the pulse. When the pressure is raised in the armlet of the Riva-Rocci or Hill–Barnard, or their modifications, or in the bag of the pocket sphygmometer (L. Hill), the pulse is supposed to disappear at the moment when the arterial lumen is obliterated, and to reappear when the patency of the channel is re-established. Consequently every effort has been made to secure that the pressure should be transmitted to the arterial wall as far as possible without loss. Accuracy in instrumental readings has been held to be conditional on such perfect transmission of pressure.

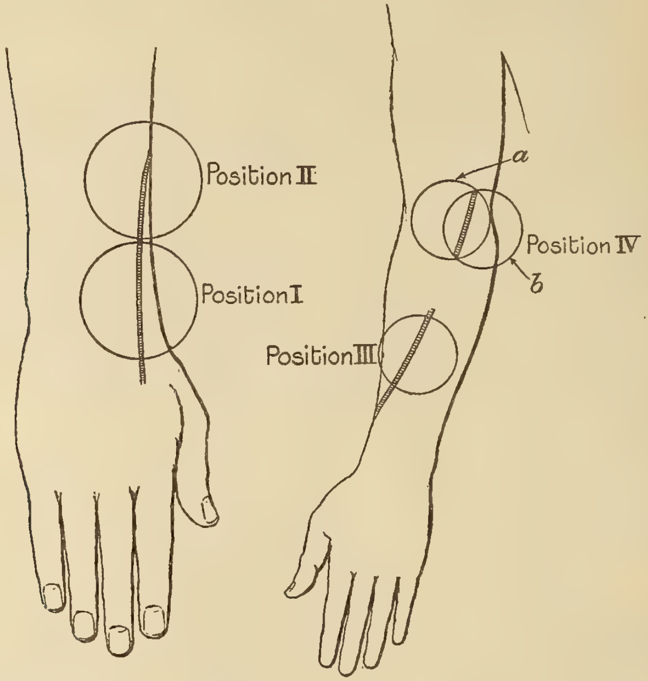
Of late years controversy has ranged round the importance of the arterial wall as a factor in blood pressure, especially in diseased conditions of the wall, *e.g.* arteriosclerosis. One of us (L. Hill) with Russell Wells (2) and Martin Flack (3) has shown the importance of the arterial wall in influencing conduction of the pulse, and has ascribed the high readings obtained in the arteries of the leg in cases of aortic regurgitation to a better conduction of the pulse in contracted and more rigid arteries. There remains for us in this paper to demonstrate another factor, hitherto overlooked, in the taking of blood-pressure observations, namely, the influence on the arterial pulse of the resonance of the tissues permeated with arterioles. The pulse is essentially a phenomenon of periodic vibrations, and by the resonance of the tissues we denote the property of the tissues to further the pulse vibrations by synchronous vibrations of like (positive) periodicity.

Our observations have been made in the first place on a man, a boiler-maker by trade, aged 53 years, whose arteries show on both arms slight though equal arteriosclerosis. His apex beat is visible within the nipple line, and his cardiac valves are intact. No aneurismal condition is detected.

His right radial artery pursues an aberrant course, curving some 3 inches above the styloid process of the radius over the supinator longus muscle on to the dorsal surface of the forearm, where it runs over the extensor tendons of the thumb, till it dips between the interossei muscles in the first interosseus space to join the deep carpal arch. The brachial artery in the arm and the radial artery in the forearm are divided by us into certain positions.

Position I (radial artery) denotes the part of the artery on the back of the hand that can be covered by the bag of the L. Hill sphygmometer while space is left for pulse observation distal to it.

Position II (radial artery) denotes that part of the artery which can be covered by the bag as it curves from the dorsal surface to the palmar surface of the forearm.



Position III (radial artery) denotes the superficial course of the radial artery in the forearm just previous to its dipping deep between the supinator longus muscle and the pronator radii teres.

Position IV (brachial artery) denotes that part of the brachial artery that lies superficially in the antecubital fossa.

In position I the artery lies superficially under the skin, and is placed upon an unyielding bed of bone, the carpal bones, their ligaments and the tendons of extensor muscles. Such an observational site may well be taken as a standard, in the light of which all other positions may be reviewed. The bag of the pocket syhygmometer applied on the artery at position I cannot fail to transmit pressure equally to all parts of the artery beneath the bag, and there can be no loss of pressure here through the faulty transmission of intervening tissues or through distortion of tissues.

Analysed anatomically position II is similar to position I, while in position III the radial artery courses over the pronatus quadratus and flexor longus pollicis. At position IV the brachial artery lies on the deep tendon of the brachialis anticus muscle.

Taking readings of disappearance and reappearance of the pulse with the pocket sphygmometer we find—

				mm. of Hg.			mm. of Hg.
Position I		pulse	disappearance	55	pulse	reappearance	50
Position II		„	„	55	„	„	50
Position III		„	„	130	„	„	125
Position IV	A	„	„	105	„	„	100
	B	„	„	75	„	„	70

Substituting a bag of water for one of air the readings are—

				mm. of Hg.			mm. of Hg.
Position I		pulse	disappearance	55	pulse	reappearance	50
Position II		„	„	45	„	„	40
Position III		„	„	130	„	„	125
Position IV	A	„	„	119	„	„	109
	B	„	„	65	„	„	63

The subject was in the horizontal position in all cases.

The low reading of 55–50 mm. of Hg at position I cannot be due to any fault in the transmission of pressure through the bag to the arterial wall Consequently, we assume that the pulse has disappeared at 55–50 mm. of Hg before the blood flow has ceased through the artery. In other words, the phenomenon of arresting the pulse by occlusion of the artery is not brought into play in this observation.

Two methods suggest themselves by means of which it can be proved that when the pulse ceases to be felt at position I the arterial flow is still maintained, that the pulse has, as it were, been skimmed off the current.

Keeping the bag of the sphygmometer pressed on position II with a pressure of 180 mm. of Hg, one can strip the blood out of the artery, and, to prevent recurrent flow, fix the artery below, as it dips through the interosseous space. By releasing the pressure at II, the lumen of the empty artery can be felt to fill with blood when the pressure in the bag registers 115–120 mm. of Hg. It can be felt standing out as a bulging cord at 90 mm. of Hg, while the pulse returns at 60 mm. of Hg.

It is possible to place the armlet so as to cover position III and part of

II, while the bag is pressed on part of II and part of I, the following reading can then be taken :—

	mm. of Hg.
Pressure in armlet	90
Pulse disappears below bag at	55

Here there can be no question that the blood flow passes through the pressure of 90 mm. of Hg, and therefore cannot be arrested by a pressure of a bag at 55 mm. of Hg. A further possibility suggests itself that the pulse may be diverted through pressure on the bag, and seek an easier channel through some branch of the radial artery. Against this supposition we suggest: first, that the branch chosen must be a big one, otherwise what the pulse gains in an easier path is lost in the friction due to the narrower lumen; second, that a pulse would never pass back from the bag at 50 mm. of Hg, under the armlet at 90 mm. of Hg. Consequently we conclude that with the bag in position I the pulse is damped down under the bag, while there is but a trifling obstruction to the blood flow in the artery. The blood in the artery below the bag takes on the characters of a venous flow.

The aberrant radial artery where it lies in part of position I, in position II, and position III was covered by the armlet, and while preventing the recurrent ulnar pulsation, a reading was taken. The pulse was then found to disappear and reappear between the limits of 120–130 mm. of Hg. Consequently the aberrant radial artery in positions I and II, overlying bone ligaments and tendons, can withstand a pressure of, say, 110 mm. of Hg without the pulse being damped down. But with the bag of the pocket sphygmometer at position I or at position II, the pulse is removed from the blood current with a pressure of 55–60 mm. of Hg. Yet, according to physical laws, the pressure is equally delivered to the elastic wall of the artery by both instruments. The problem is seen then to depend on the air contained in the armlet in the one case, and on the air contained in the bag in the other. It is not a matter solely of pressure in the air of the armlet or of the bag, but the important factor is the state of the air in both cases as regards periodic vibrations.

The air in the armlet is in a state of periodic vibration. These vibrations depend on the pulsation of the mass of tissues which surround the ulna and radius and are embraced by the armlet. At every beat of the heart the incompressible blood is pumped into the tissues through arteries large and small, and the pulse of each and every artery is directed as much outwards into the tissues as inwards upon the blood stream. Consequently the tissues become a pulsating mass, as can be registered on a plethysmograph curve. When the bag of the pocket sphgymometer is applied to the artery, either at position I or at position II, the pulsations in the air of the bag are at a minimum, because the tissues lying under the bag are comparatively pulseless. In the case of the armlet, with its wider embrace of pulsing tissues, the air shows pulsations more or less synchronous to the pulse in the artery, the arterial pulse is thereby strengthened and enabled to resist the damping-down effect of the armlet. Consequently the pressure applied to the arterial wall may be increased from 60 to 100 or 110 mm. of Hg, as the case may be, and yet the pulse persists, provided the medium through which the pressure is applied is itself in a condition of like periodic vibration. Of course, the vibrations must be of such a period as will strengthen the pulse of the artery and not oppose it.

It is on this fundamental experiment that the hypothesis of the resonance of the tissues is grounded. By this hypothesis we can explain the various readings obtained by the same instrument (*e.g.* bag of pocket syhygmometer) at positions I, II, III and IV. Position II is obviously similar to position I.

In position III the radial artery lies as we trace it centrally, first on the pronator quadratus, and then on the flexor longus pollicis. When the bag is applied to the artery in position III, there are beneath it fleshy tissues with numerous arteries in them. Consequently, the tissues below the bag are throbbing more or less synchronously with the pulse in the radial artery at position III. The air of the bag is then in a state of periodic vibration, as in the case of the air of the armlet. Accordingly, the reading becomes the high one of 130–135 mm. of Hg. The damping-down effect of the bag on the pulse has been compensated for by the resonance of the tissues beneath it. Readings with the bag of the pocket sphygmometer placed at position IV have been noted to vary from 60 to 100 mm. of Hg. In taking these readings, the recurrent ulnar pulsation can be damped down at position I, and the pulse felt at position II. Such variable readings do not occur haphazardly; it can be demonstrated that they depend on the varying anatomical condition of the areas below the bag. Such areas may be classified into areas of high resonance and areas of low resonance.

If a diagonal line is drawn through the centre of the superficial brachial artery at position IV (see Diagram), the bag of the sphygmometer can be so placed that 1/3 of the bag lies to the radial side of the artery and 2/3 on the ulnar side, or, the same length of artery being covered as before by the bag, 2/3 of the bag can lie to the radial side of the artery and 1/3 to the ulnar side.

These positions are indicated by the circle (*a*) and the circle (*b*).

It is to be noted that the same length of artery is under pressure in both cases. The bags are covered with the hand in a precisely similar manner, yet the pulse at *a* reappears at 90 mm.; at *b*, reappears at 60 mm.

This difference can be explained by an analysis of the tissues underlying the bag in either position. In position *a*, 2/3 of the bag lies on the fleshy belly of the supinator longus and biceps, and over the arterial anastomosis of the radial recurrent artery and the superior profunda artery. In position *b*, 2/3 of the bag lies on the tendinous insertions of the flexor group of muscles. Here the arterial supply is much less. Consequently, the resonance of the tissues in position *a* is greater than the resonance of the tissues in position *b*, and the pulse suffers a great damping-down in position *b*.

Here we have no question of loss of pressure through overlying or distorting tissues. The tissues over the artery are the same in both cases. The pressure on each point of the circumference of the bag is the same. Consequently, it must be that, in position *a*, the air delivering the pressure is in a state of greater periodic vibration than the air in the bag in position *b*. The vibrations that underlie the phenomenon of sound are transmitted in water as in air. We find that when water is substituted in the bag for air the same results are obtained. The water takes on the periodic vibrations of the resonating tissues.

L. Hill and Russell Wells (2) have recently shown how important a factor in the pulse curve is the lability of the arterial wall. It has also been shown by L. Hill and Martin Flack (3) that, when an artery is freed from the tissues, and thereby deprived of the support of the tissues round its wall, the pulse curve is much affected. The lability of the wall is called into play, and the systolic pressure of the heart is spent in distending the wall of the artery. It was possible, then, that the artery lying more or less superficially at positions II, III, and IV, would have its wall distended, so that the pulse arriving under the bag at position I would be already damped down before pressure was applied to the artery at position I.

Our experiments show that, at position I, with a pressure, say, of 60 mm. of Hg, the pulse is skimmed off the blood current, but the arterial flow remains. Consequently, the block on the blood flow is not an absolute one.

Experiments were made by supporting the superficial artery with the armlet and with the bag of another sphygmometer, to determine whether such support played any part in the production of the low pressure reading at position I.

Our results show that no matter what pressure is raised in the armlet on positions III and part of II, the pressure in the sphygmometer bag covering part of position II and part of position I required to obliterate the pulse remains the same. On the other hand, when the artery is, in addition, supported by varying pressures at position IV, the reading becomes 5–15 mm. higher. Simultaneous support in positions IV, III, and part of II, makes the reading at part of position II and part of position I higher by 5–10 mm. of Hg.

Table where Supporting Pressure is Applied successively at Elbow and Forearm.

	Supporting pressure of sphygmometer bag at elbow, position IV.	Supporting pressure in armlet over part of III and part of II.	Sphygmometer bag at part of I and part of II.	
	mm. of Hg.	mm. of Hg.	mm. of Hg.	
Experiment I ...	40	40	75	disappearance of pulse.
	0	0	65	,, ,,
Experiment II ...	40	40	73	,, ,,
	0	0	63	,, ,,
Experiment III ...	40	40	65	,, ,,
	0	0	50	,, ,,
Experiment IV ...	0	0	55	reappearance ,,
	0	40	55	,, ,,
	40	40	65	,, ,,
Experiment V ...	0	0	55	,, ,,
	0	40	55	,, ,,
	40	40	65	,, ,,

Table where Supporting Pressure is Applied at Forearm only.

Supporting pressure of armlet in position III and part of II.	Sphygmometer bag on part of I and part of II.	
mm. of Hg.	mm. of Hg.	
0	50	reappearance of pulse.
10	50	,, ,,
20	50	,, ,,
30	50	,, ,,
40	50	,, ,,
50	50	,, ,,
60	50	,, ,,
70	50	,, ,,
80	50	,, ,,
90	50	,, ,,
100	50	,, ,,
110	50	,, ,,

Note.—It is important to commence from zero and work upwards and not raise the pressure to 110 mm. of Hg all at once, because venous congestion, which is rapidly accommodated for when rising from zero, otherwise proves a disturbing factor.

Table where Supporting Pressure is Applied as far as possible Simultaneously at Elbow and Forearm.

Supporting pressure of armlet applied at position III and part of II.		Supporting pressure of sphygmometer bag at position IV.	Sphygmometer bag at part of position I and part of II.
			Increase in mm. of Hg on previous reading before application of supporting pressure at elbow and forearm.
	mm. of Hg.	mm.	
Experiment I ...	20	20	5–10
Experiment II ...	30	30	5–10
Experiment III ...	40	40	5–10
Experiment IV ...	50	50	5–10
		Note.—Care must be taken that the bag at IV is applied as in Diagram III *a*.	

Note.—It is not possible to apply these pressures at Positions IV, III and part of II with perfect synchronism, as the pressure cannot be raised in the armlet to 50 mm. without two compressions of the pump.

Accordingly, provided one guards against errors from change in the arterial wall through manipulation—we have noted that after many readings with the bag at position I the artery becomes obviously harder and the reading rises—and, provided one constantly guards against a rise in arterial pressure during an experiment, then lack of support of the wall may account for a loss of pressure of 5–10–15 mm. of Hg. But such lack is obviously unable to account for the low reading at position I of 50–60 mm. of Hg. Further, the experimental observations with the sphygmometer bag on varying positions at IV show that the main factor must be the resonance of the tissues.

But low readings with the sphygmometer bag are not confined to aberrant radial arteries. Thus one may observe the same phenomenon on the dorsalis pedis artery.

Reading with bag on dorsalis pedis horizontal position—

	mm. Hg.		mm. Hg.
Dorsalis pulse disappears	85.	Right radial pulse disappears	145.
„ „ reappears	80	„ „ „ reappears	140.

In this case the dorsalis pedis available was short and the foot was fleshy.

In another case where the dorsalis pedis is longer and the tissues surrounding it scantier, then—

	mm. Hg.		mm. Hg.
Dorsalis pulse disappears at	55.	Left radial pulse disappears at	135.
„ „ reappears „	50	„ „ „ reappears „	130.

Subject in the horizontal posture.

In yet another case the pulse disappeared at 35–40 mm. Hg.

The anterior tibial artery in the leg is overlapped in the upper part of the leg by the tibialis anticus muscle, in the lower part of the leg by the extensor longus digitorum, extensor proprius hallucis, and anterior annular ligament. The dorsalis pedis artery is overlapped by the anterior annular ligament and by the innermost tendon of the extensor brevis digitorum. Consequently, the artery above the point of application of the sphygmometer bag is well supported. Yet the readings are similar to readings on positions I and II of the aberrant radial artery.

The low blood-pressure readings obtained with Hill's pocket sphygmometer on the aberrant radial artery, or on the dorsalis pedis artery, are due to the absence of the resonance of the tissues. Provided one could, in the forearm, tie every artery except the radial, and every large branch of the radial artery, one would find then that the blood-pressure readings taken by Hill's pocket sphygmometer, or by the armlet method, would approximate closely to the low readings found in the aberrant radial artery.

Another method of demonstrating the effect of resonance on the pulse is the following:—Blood-pressure readings are taken in an individual in the upright position, from the forearm held at the level of the heart. The systolic blood-pressure is found to be 120 mm. of Hg (disappearing pulse index). A similar reading is found in the other arm. One arm is then fully extended above the head, and the forearm, from the tips of the fingers to the elbow, is bandaged tightly to render the limb ischæmic. An armlet is fitted to the upper arm, and the pressure is raised in it well above the systolic pressure to prevent the blood flowing into the ischæmic limb. The bandage is then removed, and the arm lowered to the heart level. Hill's pocket sphygmometer is now placed on the forearm covering the same position as before (the position is previously outlined with ink) and the radial artery is blocked with one finger to prevent a pulse from the ulnar recurrent artery; the pressure in the armlet is then let down rapidly by pulling the tube off the metal connection of the compressing bulb. When the first pulses are felt at the wrist, the bag of the sphygmometer is pressed on to the artery until the pulse is damped down, a pressure of 70 mm. of Hg suffices to do this. Soon the pulse reappears below the bag, and the bag has to be pressed on with, say, a pressure of 80 mm. of Hg before the pulse again disappears. We find the systolic blood-pressure readings rise successively from 70 mm. to 80–90–100–110–120–130–140 mm. of Hg. There may, or may not, be a rebound effect when the blood pressure rises, for a short period, above what it was at the commencement of the experiment, and above the reading in the forearm of the other arm.

The ischæmic limb on the abolition of pressure in the armlet on the upper arm is found to gradually swell and becomes red. There is obviously a marked vaso-dilatation.

Bayliss (4) has shown that when the blood pressure is taken off a limb or an organ, *e.g.* by blocking the abdominal aorta, an increase in volume of the limb or organ occurs when the block of the aorta is removed. Bayliss offers no proof as to which part of the vascular mechanism dilates in this reaction. A study of the phenomenon in a limb with an aberrant radial artery during this experiment gives a clue to the vascular conditions present in the reaction. The aberrant radial artery can be seen to dilate. It stands out like a small worm on the back of the wrist. The veins on the forearm also dilate. It is unlikely that the arterioles are constricted when there is visible an increased blush of the capillary area. We conclude that during Bayliss' phenomenon, after a bandage has been used to make the limb ischæmic, the main arteries as well as the arterioles of the limb dilate.

This can be proved by tracings taken with the Dudgeon sphygmograph from the aberrant radial artery on position I. We use weight extension to fix the Dudgeon. The base line of the tracings is seen to progressively rise as the artery dilates. Care must be taken to fix the limb effectively during this experiment.

A further proof that the main arteries are dilated can be got by plunging the congested limb into ice-cold water. After a period in the cold water the artery is felt to be very much constricted, and this is confirmed by visual examination. Massage of the artery brings it back to its original dilated condition.

A modification of this experiment, viz. releasing the artery and taking the blood pressure in the ischæmic limb as the limb fills with blood, can be performed. The ischæmic limb with an armlet on the upper arm at a pressure well above the ascertained systolic blood pressure can be plunged into ice-cold water with ice in it. After a short period the limb, withdrawn from the ice-cold water, is found to be thoroughly chilled and is dried by mopping lightly without rubbing. Rubbing might dilate the arteries. When the pressure is let down suddenly in the armlet, at first the pulse can be damped down by 40 to 50 mm. of Hg, then the pressure rises, but much more slowly, to normal or above normal. This is the important point, that the blood-pressure reading in the radial artery rises much more slowly in the cold ischæmic limb wherein the arteries are constricted than in the warm ischæmic limb wherein the arteries are dilated. At the conclusion of the experiment, when the blood pressure is back to normal, the aberrant radial artery still feels like a whipcord—highly contracted. Massage of the whipcord artery will bring it back to the worm-like condition which obtained in the congested limb.

Accordingly we can conclude that the phenomenon occurs in the dilated or in the contracted artery—it is immaterial which. Consequently the initial low blood pressures (as measured by the disappearance of pulse) on allowing the blood to enter the arteries are independent of the state of the arterial wall. They are also independent of the peripheral resistance.

Blood-pressure estimations were made on the aberrant radial artery at the close of these experiments on the warm limb (now congested) and on the cold limb.

When the systolic pressure had arisen to the normal 120–130 mm. of Hg in the forearm in the warm limb the reading obtained at position I on the dilated aberrant radial artery was 50–60 mm. of Hg. In the cold limb when the blood-pressure reading in the forearm was found to be 150–160 mm. of Hg (the same as the initial pressure in the individual tested), the constricted aberrant radial artery gave a reading of 70–80 mm. of Hg.

We conclude, therefore, that the pulse in either the dilated artery or the contracted artery can be damped down by a pressure 70–80 mm. Hg or so below normal. Experiments similar to the above, and with like result, can be performed on the dorsalis pedis artery.

We have traced in the ischæmic limb the rise in the size of the beat of the radial artery, or of the dorsalis pedis artery, or of the aberrant radial artery (at position I) using both Mackenzie's polygraph and the weight-extension method and the Dudgeon sphygmograph, and blocking the artery below to prevent the recurrent pulse. When the armlet is compressed in the upper arm and the pressure suddenly let go, one notes that the beat in the congested limb returns quicker to its normal size than in the ischæmic limb. In all cases the beat takes longer to come to normal when the weight-extension Dudgeon is used than when the tracings are taken by the polygraph. The weight-extension method of applying the Dudgeon avoids the plethysmographic effect of the polygraph (Lewis). One often finds the pulse takes a minute to return to its maximal swing, *i.e.* until the surrounding tissues are filled with blood and resonate with it.

It might be argued in the light of the fact that the return of the maximal beat is slower in the ischæmic limb than in the congested limb, that we have herein a natural explanation of the initial low pressure readings. The pulse beats in the ischæmic limb are of feeble force, consequently the bag of the sphygmometer applied to the artery naturally damps down the feeble beats. But we have shown that maximal beats, whether the artery is dilated or contracted, suffer a damping-down in the aberrant radial artery at extremely low blood pressures. Thus in one experiment, when the systolic blood pressure was taken at heart level by Hill's sphygmometer in the forearm at position III and was found to be 120–130 mm. of Hg, the returning pulse in the ischæmic limb at position III was damped down at 70 mm., and when the blood pressure rose at position III to 120–130 mm. the pulse in the aberrant radial artery at the back of the wrist at position I where maximal beats could be recorded was damped down at 50–60 mm. The feebler pulse beats in the forearm on the radial artery at position III required 70–80 mm. to damp them down. The maximal beats on the same radial artery at position I required only 50–60 mm. of Hg to damp them down. We see, in fact, that the pulse beat, no matter how forcible, can be damped down by a pressure 70 mm. of Hg or so below normal blood pressure.

It might be argued that the low blood-pressure readings obtained in this experiment represent the actual blood pressure in the radial artery, that there has been a fall of head of pressure as the blood flows into the ischæmic limb. It is not probable that the head of blood pressure would fall greatly, because the blood flows through the narrow arterioles and still narrower capillary bed. No matter whether the arterioles and capillary bed are full or empty, the resistance to the blood stream remains in the friction of the vessel walls. But blocking the radial artery below the point of measurement effectively removes the objection that there is a fall of head of pressure. It might be argued that the fall of pressure continues down the ulnar artery. But by blocking the radial artery one converts the radial artery into a side tube measuring lateral pressure from the brachial at the elbow, and the lateral pressure of the brachial artery at the elbow would not fall. Further, one can block both radial and ulnar arteries, and the pressure readings taken from the forearm of the ischæmic limb show the same progressive rise. We conclude that on suddenly lowering the pressure in the armlet the blood pressure rapidly becomes normal, and the low blood-pressure readings, as measured by the disappearance of the pulse, are false, both in the ischæmic limb and in the cold ischæmic limb.

The explanation of these low blood-pressure readings lies in the diminished resonance of the empty tissues. The mass of tissue below the bag is not tense with blood and does not vibrate strongly with the pulse, consequently the sphygmometer bag acts as a damper. The rise of the pulse to maximal is aided by the resonance of the tissues. But whether the pulse beat is maximal or not it is bound to suffer damping down so long as the resonance of the surrounding tissues is feeble.

It will be noted that the blood pressure in the cold ischæmic limb returns much more slowly to normal than in the warm ischæmic limb. Here the arterioles of the limb are contracted; consequently the blood takes longer to percolate into the ischæmic tissues, the drum-head takes longer to tighten up, and the resonating effect consequently longer to develop. After dilatation has been produced in the vessels of a limb, repeatedly made ischæmic, it is less easy to obtain the staircase effect. The bandaging has then to be done very tightly; on letting go the brachial artery the blood rushes in swiftly, the skin blushes, and the maximal beat quickly returns.

Many years ago Hürthle (5) noted that the diastolic pressures taken simultaneously with a manometer at the femoral artery and at the carotid artery were nearly similar, while the systolic pressure at the femoral exceeded that of the carotid by roughly 68 mm. of Hg. Dawson (6) corroborates this statement, working with the maximum and minimum manometer, but points out the diastolic pressure in the femoral is always slightly lower than the diastolic in the carotid.

We would advance the explanation of the higher systolic and lower diastolic readings in terms of the resonance theory. The abdomen functionates as a resonator of the pulse, because each organ in it—liver, spleen, kidney, intestines, etc.—are all pulsating and the cavity is a closed one. Descent of the diaphragm is compensated for by an outward movement of the abdominal wall. The abdominal wall is an elastic structure. Consequently the systolic pulse in the aorta and great vessels is surrounded by more or less synchronous pulsations, which, like the well adjusted tap on the moving pendulum, augment its swing.

In the case of the higher blood-pressure readings in the leg arteries, compared to the arm readings found by Hill, Flack, Holtzman and Rowlands (1) in cases of aortic disease, we believe the same resonating effect of the abdominal cavity is at work, together with the better conduction of the pulse wave down the tighter abdominal and leg arteries.

It was suggested by one of us (L. Hill) in 'Further Advances in Physiology' (7), that the kidney functionated largely through the mechanism of the arterial pulse. "In the case of the kidney the blood in the capillary network, the tissue lymph, and the urine in the tubules are all at one and the same pressure—the capillary-venous pressure. The whole kidney is expanded by each arterial pulse, and drops of urine may be squeezed thereby into the pelvis from the mouths of the tubules." Recent work by R. A. Gesell (8) has shown that the excretion of the urine, the chlorides, urea and nitrogen is dependent on the arterial pulse. It is to enable the pulse to be driven to the capillary areas in the kidney or other organ that the mechanism of a resonation of the tissues is called for. Without some such mechanism the pulse would be inevitably damped down, especially during the varying abdominal pressures found with deep inspiration, forced expiration, defæcation, etc.

Further, we would advance the view that by abdominal resonance the pulse wave is assisted to the most distant peripheral regions of the body. The aortic pulse finds its way to the tips of the fingers in aortic disease and to the toes. The longer path is compensated for by abdominal resonance.

Resonation of the tissues must be held to play an important part in the transmission of the pulse, and thereby to save the work of the heart. The work of the heart we know is largely conserved by the elastic recoil of the arteries. But this elastic recoil of the arteries is aided by the resonance of the tissues. Every artery is in intimate relationship with its immediate neighbour. The pulse of one individual artery is aided by the pulses of the other arteries. The vigour of the circulation depends on the tone of the tissues, on the tautness of skin and muscle, and particularly of the abdominal wall. The hardened body of the trained athlete swings in full resonance with the pulse of his heart; the soft, flabby, ill-conditioned body of the sedentary worker offers a poor slack drum for his heart to thump.

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On a Method of Studying Transpiration.

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(Received October 22,—Read December 4, 1913.)

Transpiration is, perhaps, more directly under the rule of external physical conditions than any other physiological function. Yet proofs of this conclusion are wanting, at any rate in regard to the transpiration of leaves.

Thus, as far as I know, we have no complete experimental determination of the relation between the loss of water-vapour from leaves and the relative humidity of the air. Nor again have we any complete evidence as to the effect on transpiration of variation in the illumination to which the leaf is subjected.

These lacunæ in our knowledge depend on the fact that in leaves, transpiration is largely dependent on the behaviour of the stomata, being relatively large when they are wide open, and diminishing as they close. And since the aperture of the stomata depends on external condition, it is clear that no distinction can be made between the diminution in evaporation resulting from increased relative humidity of the air, and the diminution in the transpiration-rate due to stomatal closure. In fact it is impossible to learn anything accurately concerning transpiration until the varying aperture of the stoma is excluded from the problem. This might possibly be done by estimating the transpiration of leaves of aquatic plants in which the stomata vary but slightly in aperture; but the experiment would not be easily made in a trustworthy form.

The method I have actually employed is to block the stomata with a fatty substance,* and then to place the intercellular spaces of the leaves in communication with the external air by means of incisions.

Most of the experiments were made on laurel (*P. laurocerasus*). The lower surface of the leaf was smeared with melted cocoa-butter or with vaseline rubbed in with the finger, and four to six cuts were made with scissors or a razor, reaching from the periphery to the midrib between the large veins. Other plans were also tried, *e.g.* pricking the leaf with a needle or making numerous small incisions by stabbing with a scalpel.†

The method is similar to that of Stahl,‡ who showed that greased leaves pierced with holes assimilate and form starch in the tissues surrounding the wounds, whereas greased leaves without such artificial stomata formed none or hardly any.

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* Cocoa butter in the earlier experiments, vaseline in all the later ones.

† The method was described in a paper read at Section K of the Sheffield meeting of the British Association, 1910 (title alone published).

‡ 'Bot. Zeit.,' 1894.

It may be objected that the stomata are not completely or uniformly closed by greasing, that some remain open, and that it is to the opening and closing of these in light and darkness that the rise and fall of the transpiration of the incised leaves is due. I find it difficult to believe that the general objection here discussed is sound, because experiments with the porometer* have convinced me that even a careless application of vaseline absolutely closes the stomata. It may be urged that in Experiment LO 2 (p. 271) the effect of grease is only to reduce transpiration from 379 to 10·9, *i.e.*, from 100 to 2·88. It must be remembered, however, that fatty substances are not impermeable to water, and that at any rate part of the 2·88 per cent. must be due to cuticular transpiration.

Another source of error should be guarded against. Mr. Blackman† has shown that a process of healing occurs in wounded laurel leaves. The beginning of the process is, however, marked by the edges of the wounds becoming translucent. As soon as this occurs the specimen should be discarded or fresh incisions made.

The following experiment, LO 2, October 2, 1912, gives an idea of the effect of greasing and slitting. It seems clear that the result is comparable (as far as magnitude is concerned) with normal stomatal transpiration :—

Experiment LO 2. October 2, 1912. *P. laurocerasus.*

A laurel branch cut under water with 10 leaves (one being small) having a stoma-bearing area of 600 cm.^{2}.

Fitted to a potometer (diameter of tube 0·95 mm.). At a north window, where the temperature during the observations varied between 13·6° and 15·2° C. and the relative humidity between 59 and 69 per cent. In the following abbreviated record of the experiment the potometer readings are corrected for differences in relative humidity.

A.M.	Transpiration.
11.10	439‡
27	427
53	379
P.M.	
12.18	Finished vaselining leaves on both surfaces.

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* See F. Darwin and D. F. M. Pertz, 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 137, for a description of the porometer.

† F. Blackman and G. Matthæi, 'Annals of Botany,' 1901, vol. 16.

‡ The figure 439 is obtained from the number of seconds (viz. 22·8) in which the column of water in the potometer tube travels 1 cm., which means the absorption of

P.M.		Transpiration.
12.20		285
34		64·7
43	Surface of branch vaselined.	
59		47·3
4.36		18·1
Oct. 3—		
A.M.		
10.23	Fresh surface cut to branch.	
11.21		10·9
32	Four incisions made per leaf, *i.e.* two on each side of midrib.	
36		94·3
P.M.		
12.5	One more slit per side.	
7		196
12	One more slit, making four per side	
44		255
Oct. 4—		
A.M.		
10.11	Fresh surface cut to branch.	
11.5		234

It will be seen that the coating of vaseline on the leaves and surface of the branch does not completely check transpiration. Thus, as above mentioned, on the second day (October 3), when the original negative pressure must have disappeared, transpiration had only been reduced from 379 to 10·9 or from 100 to 2·88. This fact is in the present instance of little importance, as my object is to illustrate the effect of incisions on the transpiration rate.

It is obvious (i) that when the lamina is cut into strips the transpiration rises with great rapidity; (ii) that although in this instance it does not obtain the rate of transpiration observed when the stomata were open, the two are comparable for practical purposes.

In the case of these slit leaves it is of some interest to know the amount of connection between the external air and the intercellular spaces. This was estimated from the observations on the laurel twig (Experiment LO 2) just described. Each leaf had eight incisions (four per side), varying in

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0·00708 c.c. The figure 439 is the reciprocal of 22·8 multiplied by 10,000. To convert the number 439 into cubic centimetre it is only necessary to multiply it by 2·55 mm.^{3} which gives the rate, in this case 1·12 c.c. per hour per 600 cm.^{2} or 18·7 c.c. per square metre of stoma-bearing area.

length from about 25 to 40 mm. The sum of the lengths of the incisions = 2437 mm. The thickness of the leaves was taken as 0·38 mm., and since each incision exposes two leaf-sections to the air, the total area of section exposed by the experiment is

$$2 \times 2437 \text{ mm.} \times 0{\cdot}38 \text{ mm.} = 1852 \text{ mm.}^2 = 18{\cdot}52 \text{ cm.}^2.$$

The stoma-bearing area of the 10 leaves, omitting the mid-ribs, was 600 cm.^{2}, so that the amount of surface exposed by incision is 18·52 per 600 or 3·09 per cent. Unger* gives for *P. laurocerasus* the intercellular spaces as 21·9, say, 22 per cent.† of the volume of the leaf. Therefore of the transverse section exposed by incision only 22 per cent. is intercellular space. We may therefore say that in a laurel leaf having four incisions on each half of the lamina the transpiratory apertures connecting the intercellular spaces with outer air are $22 \times 3{\cdot}09/100$ or 0·68 per cent. of the area of the leaf. Since these correspond in function to stomata it is worth while comparing them with actual stomata.

A rough calculation gave the area of the laurel stomata as 0·88 per cent. of that of the leaf. The transpiring area of the slit leaves is, therefore, much the same as that of the stomatal apertures under ordinary conditions.

The Effect of Changes in the Humidity of the Air.

The method of incision has been used in studying the effects, on transpiration, of variations in the relative humidity of the air; and this has led to a rough plan for reducing transpirations at varying humidities to a common standard. The method of producing a damp atmosphere was a simple one. At first the plant was covered with a large bell-jar resting on a ground-glass plate, and so arranged that a current of air, dry or moist, could be drawn through it. But finally I came to the conclusion that a simpler method was preferable, namely, to change the relative humidity by raising or lowering the bell-jar; in this way—assuming that the laboratory air is fairly dry—it is easy to change the relative humidity from 50 per cent. to 95 per cent., which is sufficient for my purpose.‡ The wet and dry bulb thermometers were in the upper part of the jar, while the branch had leaves in both lower and upper regions. I did not find this to be a serious source of error, and it is one which might be avoided by fitting an apparatus by which the air in the bell jar could be stirred and thoroughly mixed, as indeed was done in some of the later experiments.

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* 'Sitzb. K. Akad. Wien,' 1854, vol. 12, p. 367.

† Microscopic examination of a transverse section led me to estimate the air spaces as roughly 25 per cent.

‡ It is unfortunate that these observations, with the exception of Experiment 8, were not made in darkness or in constant light. The experiments which are most likely to be vitiated by this fault are Nos. 3, 4, and 7. Experiment 4 might be expected to give an especially bad result from the effect of dull light at the end of the experiment. But the diagram, fig. 4, shows rather striking uniformity in the relation between transpiration and humidity of air. In Experiment 3 the diagram is not very satisfactory in any case, but omission of the last two readings (the ones under suspicion) would not alter the

The rate of transpiration was estimated by a potometer, not one of the type formerly used by me,* in which an air bubble is timed as it passes rapidly along a narrow capillary tube, but one in which the free end of the water-column is timed with a stop-watch as it passes along a horizontal tube of about a millimetre internal diameter.† It is, in fact, like Kohl's potometer, or that figured in Pfeffer's 'Physiology,' though the method of bringing the column back to zero is not identical with either. I have not thought it necessary to give the actual quantities of water absorbed by the plant per hour, but merely a series of numbers proportional to the rate of absorption.

In all experiments (except No. 8) the plants were placed close to the north windows of the laboratory; the action of the stomata was in all cases excluded by a coating of grease, transpiration taking place only by incisions, as above described.

In the following tables T means temperature, ψ stands for relative humidity:—

Experiment 1.—November 6, 1909. *P. laurocerasus.* Fig. 1. Cut branch in potometer.

Time.	Period.	Rate.	T.	ψ.
			° C.	per cent.
10.13 A.M.	i	36	13·8	74
10.39 „	ii	38	13·6	74
10.49 „	iii	36	13·6	74
	Bell-jar over plant.			
11.16 „	iv	16	14·2	91
11.28 „	v	20	14·7	92
11.37 „	vi	14	15·0	93
11.51 „	vii	17	15·2	94

transpiration curve. Experiment 7, in which the last reading was taken at sunset, gives, nevertheless, a good straight diagonal, as seen in fig. 7. A number of experiments were made (like Experiment 8) in the dark room. I cannot see that they differ as a whole from those illustrated in the present paper.

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* F. Darwin and R. Phillips, 'Camb. Phil. Soc.,' 1886, vol. 5; see also F. Darwin and Acton, 'Physiology of Plants,' 1901, 3rd Edit., p. 79.

† In all the later experiments the diameter was either 0·95 mm. or 1·1 mm.

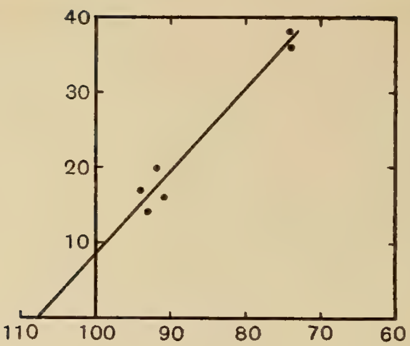

Fig. 1 (Experiment 1).

In the figures the ordinates represent transpiration rates, while the relative humidity (ψ) is given on the horizontal axis. Thus if transpiration varies directly as the relative humidity, the diagram should give a straight diagonal line. The fact that the diagonal does not pass through the intersection of the axes will be discussed later.

Experiment 2.—November 8, 1909. *P. laurocerasus.* Fig. 2.

Time.	Period.	Rate.	T.	ψ.
			° C.	per cent.
11.48 A.M.	i	56	15·8	53
12.3 P.M.	ii	56	15·4	57
	Bell-jar over plant.			
12.24 ,,	iii	35	15·1	89
12.35 ,,	iv	32	15·2	92
12.44 ,,	v	27	15·4	93
2.7 ,,	vi	17	15·6	94

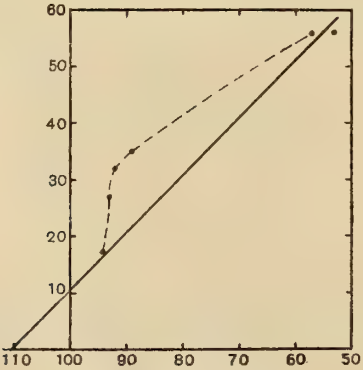

Fig. 2 (Experiment 2).

It will be seen that the dots representing transpiration for various values of ψ are by no means in a straight line. This I take to be "lag," that is to say, a relatively slow response to the change in the humidity of the air (ψ). When the air is drying instead of becoming damper the "lag" is of the opposite character, as seen in fig. 6.

Experiment 3.—November 15, 1909. *P. laurocerasus.* Fig. 3.

Time.	Period.	Rate.	T.	ψ.
			° C.	per cent.
11.30 A.M. 12.6 P.M.	i	57·0	15·4	54
12.12 „	Bell over plant.			
12.19 „ 12.44 „	ii	50·0	15·4	60
2.54 „ 3.19 „	iii	23·5	15·4	83
3.28 „	Water poured on floor of bell.			
3.44 „ 3.56 „	v	13·0	15·5	94
3.56 „ 4.10 „	vi	11·0	15·4	94

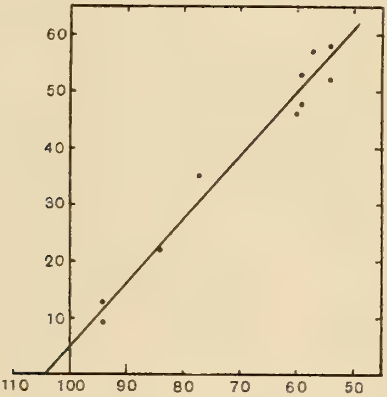

FIG. 3 (Experiment 3).

Experiment 4.—January 1, 1910. *P. laurocerasus.* Fig. 4.

Time.	Period.	Rate.	T.	ψ.
			° C.	per cent.
12.35 P.M.	i	42·7	14·4	53
12.51 „	Bell-jar on.			
12.59 „	ii	37·9	14·6	63
2.30 „	iii	33·3	14·2	61
3.12 „	iv	30·7	14·7	68
3.22 „	v	25·8	14·8	72
3.35 „	vi	22·6	14·7	77
3.47 „	vii	19·2	14·7	81
4.4 „	viii	16·1	14·9	89

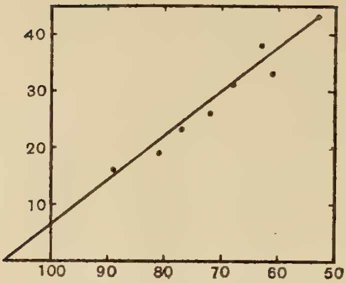

Fig. 4 (Experiment 4).

Experiment 5.—January 3, 1910. *P. laurocerasus.* Fig. 5.

Time.	Period.	Rate.	T.	ψ.
			° C.	per cent.
10.33 A.M.	i	39·4	16·3	61
10.57 „	ii	29·6	15·8	72
11.52 „	iii	28·1	16·0	74
12.19 P.M.	iv	22·3	16·4	78
12.37 „	v	21·3	16·4	82
12.46 „	vi	17·2	16·4	87
1.33 „	vii	16·5	16·0	85
1.40 „	viii	14·3	16·1	89
1.54 „	ix	10·6	16·1	94

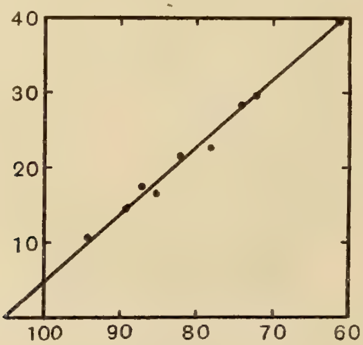

Fig. 5 (Experiment 5).

During the above observations the bell-jar had been gradually lowered, *i.e.* the supports replaced by smaller ones until only a crack, 1 or 2 mm. in height, remained. The bell was now (1.57) raised to 7 mm. and a current of air drawn through. The supports were gradually increased in height and finally (3.20) the bell was removed altogether.

Experiment 6 (= 5 continued).—Fig. 6.

Time.	Period.	Rate.	T.	ψ.
			° C.	per cent.
1.54 P.M.	i	10·6	16·1	94
2.24 ,,	ii	20·8	16·3	78
2.39 ,,	iii	24·7	16·3	72
3.6 ,,	iv	30·3	16·5	69
3.17 ,,	v	34·6	16·2	67
3.29 ,,	vi	41·7	17·0	59
3.33 ,,	vii	43·5	17·0	58

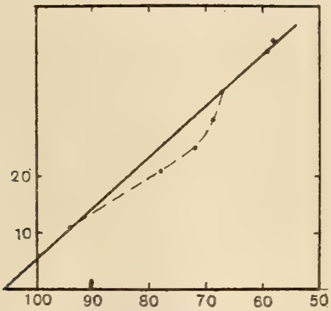

Fig. 6 (Experiment 6).

In Experiment 6 the air is drying instead of becoming damper, and the "lag" is of an opposite character to that in fig. 2.

Experiment 7.—November 15, 1909. *P. laurocerasus.* Fig. 7.

Time.	Period.	Rate.	T.	ψ.
			° C.	per cent.
11.30 A.M. 12.6 P.M.	i	57·0	15·4	54
12.12 P.M.	Bell on.			
12.19 ,, 12.44 ,,	ii	50·0	15·4	60
2.54 ,, 3.19 ,,	iii	23·5	15·4	83
3.28 ,,	Water on floor of bell.			
1.44 ,, 1.56 ,,	iv	13·0	15·5	94
3.56 ,, 4.10 ,,	v	11·0	15·4	94
	Bell removed.			
5.14 ,, 5.36 ,,	vi	54·0*	15·9	50

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* The value of this observation is doubtful; it is marked with a × in fig. 7 and omitted drawing the diagonal.

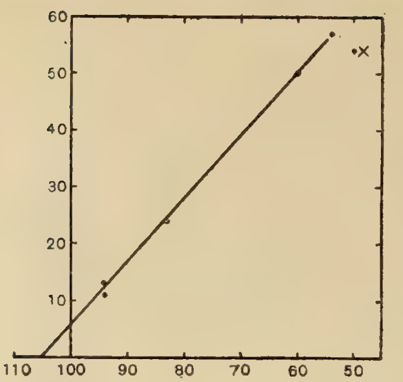

Fig. 7 (Experiment 7).

Experiment 8.—April 22, 1912. *P. laurocerasus.* Fig. 8. Apparatus fitted up in the Dark Room.

Time.	Period.	Rate.	T.	ψ.
			° C.	per cent.
10.45 A.M. to 11.30 „	i	22·4 (average)	17·3	60
11.32 „	Bell jar over plant supported on blocks 25 mm. high.			
11.56 „	ii	15·1	17·8	74
12.8 „	Blocks reduced to 2 mm.			
12.14 p.m.	iii	12·7	18·0	77
12.24 „	iv	10·7	18·0	84
12.32 „	v	8·0	18·1	88
12.37 „	vi	7·8	18·1	88
12.44 „	vii	6·5	18·1	91
12.54 „	viii	5·3	18·2	93
12.59 „	ix	5·0	18·2	94
2.15 „	x	3·5	18·2	97
2.29 „	xi	3·3	18·3	98

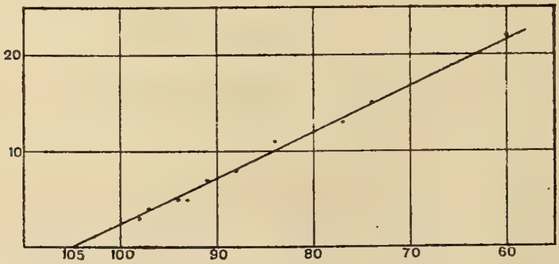

Fig. 8 (Experiment 8).

The general characteristics of the illustrations above given are

(1) The points which represent the transpiration for different degrees of relative humidity are roughly in a straight line—from which it follows that a definite relation of some sort exists between transpiration and relative

humidity. This conclusion, which is a physical necessity, does not seem to have been definitely proved or represented diagrammatically.

In some cases (*e.g.* figs. 2 and 6) the line of dots (*i.e.* the transpiration curve) is not straight—the change in rate of transpiration lags behind the change in ψ—for reasons not yet clear.

(2) The second characteristic of the diagrams is that the diagonal does not pass through the point of intersection of the axes—or, in other words, transpiration is not zero in saturated air. I have not hitherto seen this graphically represented as the result of experiment, although it might have been foretold. The fact that transpiration occurs in saturated air, and that it is due to the production of heat in plant-respiration was first made clear by Sachs*, who proposed that the fact should be utilised as a means of measuring the "Eigenwärme" of plants.† We shall see later that the diagram (fig. 9) may perhaps be applied to the same end. The position of the point G varies in different cases. In the earlier experiments, I estimated AG = 7, but I now consider 5 a more reasonable average. The construction here given has been used throughout my work for the rough reduction of transpiration-rates to a common degree of relative humidity. Thus, supposing that in fig. 9 the transpiration-rates DC and EB have been obtained under different conditions of illumination, it is clear that we cannot estimate the effect of such conditions until the amounts have been corrected for the differences in relative humidity.

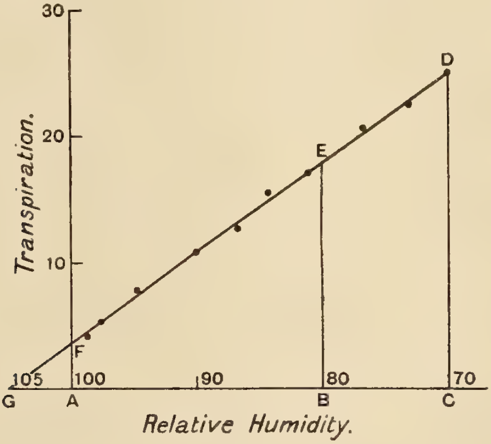

Fig. 9.

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* 'Sitzb. K. Akad. Wien,' 1857, vol. 26, p. 326.

† See Sachs, 'Physiologie Expérimentale,' 1868, p. 249 (the French translation of his book on plant physiology).

Now

$$\frac{DC}{EB} = \frac{CG}{BG} \quad \text{or} \quad DC = \frac{CG}{BG} \times EB. \quad \therefore \quad DC = \frac{105-70}{105-80} \times EB = \frac{35}{25} EB.$$

We will suppose that in an experiment on the effect of illumination we find the transpiration-rate in the light (relative humidity 70 per cent.) to be 120; while the rate in the dark (humidity 80 per cent.) is 75. We must multiply 75 by 35/25. The product 105 is the transpiration in the dark room (humidity 80 per cent.) reduced to humidity 70 per cent., and therefore now comparable with transpiration in the light, *i.e.* 120. Thus

$$\frac{\text{Transpiration in light}}{\text{Transpiration in dark}} = \frac{120}{105} = \frac{114}{100}.$$

Sir Joseph Larmor has been good enough to point out to me that it is possible to get a rough idea of the temperature of the leaf at full saturation, *i.e.* of the leaf temperature which in fig. 9 produces the amount of transpiration (or what may be perhaps called distillation) equal to AF. The oblique line, or curve of transpiration, cuts the horizontal at 105, *i.e.* at 5 per cent. above saturation. The figure shows that, in supersaturated air, *i.e.* 5 per cent. above saturation, transpiration is nil. The hypothetical degree of supersaturation should be a measure of the transpiration AF at the saturation point, and therefore of the internal temperature which can distil off water in saturated air. Assuming* the temperature of the air to be 16° C., the vapour pressure would be 13·51. If we add 5 per cent. to this we get 14·2, which is the vapour pressure corresponding to 16·8°, or 0·8° C. above the temperature of the air. There seems no improbability in leaf-respiration producing, under the conditions of the experiment, a temperature of roughly 1° C. above that of the atmosphere. In my earlier experiments I concluded that the transpiration curve DEFG cut the horizontal at 107. This would have given a temperature 1·1° C. above that of the air, instead of 0·8° C.

It should be noted that the distance AF, *i.e.* the amount of transpiration in saturated air, will depend on the general temperature, since respiration is greatly influenced by temperature. We have some evidence on this point, but the experiment needs careful repetition.

It is remarkable that, as far as I know, the method here used for plotting the relation between transpiration and relative humidity has not been employed. If Le Clerc† had treated his results in this way, he might, perhaps, have obtained a result like mine.

I cannot conclude without expressing my indebtedness to Miss D. F. Pertz for much kind help in the laboratory.

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* The figure is a diagram not taken from any one experiment.

† Le Clerc, 'Ann. Sci. Nat.,' 1883, vol. 16.

The Effect of Light on the Transpiration of Leaves.[*]

By Sir FRANCIS DARWIN, F.R.S.

(Received October 22,—Read December 4, 1913.)

The method employed is essentially that described in my paper[†] "On a Method of Studying Transpiration," where it was applied to the investigation of the relation between the relative humidity of the air and the loss of water by leaves. The stomata of the plants used were closed by vaseline or cocoa-fat rubbed in, and the leaves were then incised to allow of transpiration. No attempt was made to subject the plants to light of known intensity. My object was to compare the transpiration occurring in a dark room with that in a north light at a laboratory window. The rates of transpiration were estimated either by weighing or by means of a potometer, and the general plan was to subject the plant to alternate light and dark periods of something like an hour.[‡] The psychrometric condition of the laboratory air and that of the dark room was estimated by the wet and dry bulb thermometer, and the transpiration rates corrected for any differences, in the manner described in the paper above referred to.

The first experiment was made by a plan which has some merits, but was afterwards replaced by the simpler method of moving the apparatus from the window to dark room and back again to the light.

Experiment 1.—December 9, 1909. *P. laurocerasus.*

Branch fitted to potometer December 8 and the lower surfaces of the leaves greased; leaves cut about 10 A.M., December 9.

Placed under a bell-jar through which a current of laboratory air is drawn to keep the relative humidity (ψ) as constant as possible. The rates of transpiration are given as corrected. North light.

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[*] It is not easy to find any recorded experiments on the transpiration of leaves in light and darkness, in which the action of the stomata is absolutely excluded. In Bonnier and Mangin's experiments on the transpiration of fungi this is *ipso facto* the case (see 'Ann. Sc. Nat.,' 1884, vol. 17, p. 298). The average of the experiments on *Trametes suaveolens* is :—L/D = 119/100. For *Polyporus versicolor* the corresponding fraction is 127/100. The symbol L/D stands for the relation between the transpiration in light and darkness.

[†] 'Roy. Soc. Proc.,' this vol., p. 269.

[‡] A few weighing experiments were, however, made on the effect of the natural darkening occurring at night. The average of eight experiments gave the proportion between transpiration in the day (L) and in the evening (D), as L/D = 129/100. Four experiments made with the potometer under similar conditions gave day (L)/evening (D) = 112/100. This subject, including the effect of continuous darkness, requires fresh investigation.

Time.	Rate corrected.	T.	ψ.
		° C.	per cent.
10.42 A.M.	24·8	14·2	65
10.54 „	24·7	14·2	64
11.1 „ 11.5 „	Covered bell-jar with a black bag.		
11.21 „	27·1	14·6	62
12.5 P.M.	22·1	14·6	63
12.30 „	Light: cloth bag removed.		
12.41 „	24·9	15·2	62
2.46 „	25·8	15·7	63
3.10 „	28·0	15·7	63
3.27 „	Dark: cloth bag replaced.		
3.43 „	25·0	15·9	63
3.57 „	25·4	15·8	63
4.11 „	26·0	—	—

Result.—The fall in transpiration-rate between 11.21 A.M. and 12.5 P.M. is 27·1 to 22·1 or 123/100. The rise in the next period is from 22·1 to 28·0 or 100/127; the diminution in the final dark period is 108/100. The average proportion between the transpiration in light and darkness (L/D) is 119/100.

Experiment 2.—April 11, 1911. *P. laurocerasus.*

Potometer: T 15·0–16·2° C. ψ 46–56 per cent. Transpiration corrected.

Time.	Rate.	Time.	Rate.
11.17 A.M.	164	11.55 A.M.	107
11.33 „	140	11.58 „	103
11.37 „	122	12.13 P.M.	108
11.41 „	116	12.28 „	95
	In dark room.	4.10 „	109
11.45 „	115	4.12 „	110

Transpiration was falling (in the light) from 11.17 to 11.41; the effect of darkness was to diminish rather than to increase the rate of fall. The total change in the dark is a fall from 115 to 110, or L/D = 105/100.

Experiment 3.—April 19, 1911. *P. laurocerasus.*

Potometer: leaves slit at 10.55 A.M. T 16·1–17·0° C. ψ 50–62 per cent. Transpiration corrected.

Time.	Rate.	Time.	Rate.
11.22 A.M.	159	11.55 A.M.	186
11.39 ,,	149	12.8 P.M.	168
11.45 ,,	153	3.8 ,,	187
11.50 ,,	In dark room	3.12 ,,	181

The transpiration had been steady for some time before the plant was placed in the dark room. The only clear effect was a *rise* in transpiration-rate from 153 to 181, or L/D = 100/118.

Experiment 4.—April 20, 1911. *P. laurocerasus.*

Potometer: leaves greased and slit 10.30 A.M. T 15·0–16·6° C. ψ 44–47 per cent.

Time.	Rate.	Time.	Rate.
10.40 A.M.	757	12.2 P.M.	797
11.0 ,,	814	12.43 ,,	610
	Dark room.	12.45 ,,	Light.
11.4 ,,	902	12.48 ,,	493
11.24 ,,	897	12.56 ,,	550
11.51 ,,	816	3.30 ,,	559

In this experiment the effect of the dark room is doubtful, as the rate was not steady before darkness. If we assume that the fall in rate was due to darkness, we have the big effect of fall from 902 to 493 or 183/100. The subsequent rise in the light is from 493 to 559 or D/L = 100/113.

The average of the light and dark effects is L/D = 148/100.

Experiment 5.—April 22, 1911. *P. laurocerasus.*

Potometer: leaves greased and slit 10.31 A.M. T 19·6–21·1° C. ψ 41–52 per cent.

Time.	Rate.	Time.	Rate.
10.47 A.M.	210	11.54 A.M.	157
10.50 ,,	233	12.5 P.M.	162
10.52 ,,	208	12.13 ,,	154
10.58 ,,	213	12.21 ,,	162
11.0 ,,	In dark room	12.32 ,,	171
11.21 ,,	197	12.39 ,,	174
11.47 ,,	176	12.50 ,,	174
11.48 ,,	In light		

Transpiration was approximately steady before darkening and fell from 213 to 175, or from 121 to 100, during actual darkness; or, if we include the reading taken at 11.54, it fell from 213 to 157, or from 136 to 100.

There is the same doubt about the effect of subsequent illumination. If we compare the end of the dark period with the last reading taken in the light the effect is nil. If we compare reading at the beginning of the light (157) with that at the end (174) we get a rise of 100 to 111.

On the whole it is fairest to take the darkening effect as 136 : 100, the light as 100 : 111. The average of the light and dark effects is L/D = 124/100.

Experiment 6.—November 15–16, 1911. *P. laurocerasus.* Potometer.

Time.	Rate.	Time.	Rate.
Nov. 15.		11.30 A.M.	Light—at east window.
10.25 A.M.	Leaves slit.	11.40 ,,	146
11.18 ,,	In dark room.	11.54 ,,	134
Nov. 16.	,, ,,	12.1 P.M.	137
10.17 A.M.	Cut fresh surface to branch.	12.25 ,,	143
10.54 ,,	119	12.40 ,,	148
11.16 ,,	120	12.50 ,,	162
11.27 ,,	126	12.57 ,,	153

The effect of light may be taken as increasing the rate from 126 to 158 (the average of last two readings), or L/D = 125/100.

Experiment 7.—November 17, 1911. *P. laurocerasus.* Potometer.

Time.	Rate.	Time.	Rate.
10.15 A.M.	Leaves cut.	11.16 A.M.	In dark room.
10.18 ,,	192	11.28 ,,	317
10.34 ,,	316	11.40 ,,	292
10.39 ,,	325	12.0 NOON	294
10.50 ,,	300	12.37 P.M.	278
11.12 ,,	330	12.38 ,,	286
11.15 ,,	308	12.56 ,,	278

Shortly before the period of darkness the rate may be taken as = 320 (average of last two readings), at the end of the dark period it is 280 (average as above); this gives a diminution in transpiration equal 114 to 100, or L/D = 114/100.

The result of the above series is given in the following table; L→D means that darkness followed light, D→L indicating the opposite. The last column gives the effect as a percentage. Where, as on April 20 and April 22, there is a L→D as well as a D→ effect, the average is given :—

Experiment.	Date.		Effect of light or of dark.	
1	Dec. 9, 1909 ...	L → D	123 → 100	19 per cent.
		D → L	100 → 127	
		L → D	108 → 100	
2	Apr. 11, 1911 ...	L → D	105 : 100	5 ,,
3	,, 19, 1911 ...	L → D	100 : 100	0 ,,
4	,, 20, 1911 ...	L → D	183 : 100	48 ,,
		D → L	100 : 113	
5	,, 22, 1911 ...	L → D	136 : 100	23 ,,
		D → L	100 : 110	
6	Nov. 15, 1911 ...	D → L	100 : 125	25 ,,
7	,, 17, 1911 ...	L → D	114 : 100	14 ,,
	Average	L/D = 119/100.		

In some cases transpiration is but slightly affected by darkness, as in the ollowing experiments.

The material was supplied by small branches of laurel (*P. laurocerasus*), having, as a rule, four leaves, vaselined and cut (four incisions per leaf) in the usual way. A branch was fitted to a simple form of potometer consisting of a pipette graduated to 0·01 c.c. The pipette was fixed vertically and the branch attached to the lower end by rubber tube; as the plant absorbs water the descent of the meniscus is read with a lens, by which means errors of parallax are fairly well avoided.

The experiments were made alternately in a dark room and at the north or east window of the laboratory.* Readings were generally continued for an hour before the change from light to darkness, or *vice versâ*, was made. The results, *i.e.* the amounts of water absorbed per hour in light and darkness were corrected for psychometric differences. The dates of the experiments summarised below were April 21, 22, 23, 28, 29, May 1, 2, 3, 1913.

The results were somewhat irregular and are therefore given in the form of an average. A single experiment is, however, given in detail.

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* In a few cases in a dark room which could be illuminated by opening the shutter. The room was to the south and care was taken to avoid sunshine.

Experiment 8.—April 28, 1913. *P. laurocerasus.*

Leaves, five in number, vaselined and cut into strips at 9.40 A.M. Vertical potometer, 60 cm. from window of dark room, shutter open. T 15·2–16·2. ψ 63–65 per cent. Transpiration corrected for ψ. Dull day.

Time.	Reading.	Rates per hour.
	c.c.	c.c.
9.51 A.M.	0·080	
10.0 „	0·103	0·156
10.15 „	0·142	0·152
10.34 „	0·190	0·151
10.45 „	0·217	0·147
10.50 „	Shutters closed.	Dark.
10.50 „	0·233	0·192
11.0 „	0·254	0·126
11.15 „	0·292	0·152
11.30 „	0·326	0·136
11.45 „	0·361	0·140
	Shutters opened.	Light.
12.0 NOON	0·394	0·132
12.15 P.M.	0·430	0·144
12.30 „	0·463	0·132
12.45 „	0·497	0·136
12.46 „	Shutters closed.	Dark.
2.30 „	0·715	0·125

I have usually estimated the transpiration by taking the average of the two last readings in each period, Light (L) or Dark (D), as the case may be. But in Experiment 8 the first L reading should clearly be the average of the last L and the first D reading, *i.e.* 170. The other averages are D 138, L 134, D 125; they are included in the general average.

The results of the above-named eight experiments show considerable irregularity and no clear impression is gained by inspection. I have therefore taken the average of 31 readings from the series, 18 representing transpiration in light, and 13 in dark. They are as follows:—

Light.	Dark.	Light.	Dark.
153	155	134	125
214		200	157
186	183	161	141
216		224	181
170	166	212	152
130	122	170	138
120		149	
96	118	110	100
170	138	104	

Average 162 : 144 }
or 113 : 100 } L/D = 113/100.

Another series of similar experiments was made by Miss Pertz on *P. laurocerasus*, using a Ganong potometer.*

The following example shows a definite light and dark effect, in spite of a good deal of irregularity. The figures are corrected for relative humidity, which varies between 56 and 60 per cent., while the temperature lay between 15·5° and 16·1° C.

Experiment 9.—May 3, 1913.

Time.	Transpiration.	Average.	Time.	Transpiration.	Average.
	Light.				
10.49 A.M.	150		12.0 NOON	Light.	
11.1 „	163		12.5 P.M.	157	
11.11 „	167		12.10 „	144	
11.26 „	164	170	12.15 „	133	
11.35 „	176		12.20 „	142	149
	Darkness.		12.35 „	156	
11.43 „	172				
11.50 „	139	138			
11.59 „	137				

In the third column is given the average of the last two readings in the three periods Light, Dark, Light; thus the result of the experiment of May 3 is L 170, D 138, L 149.

The following table gives the results of the series, May 3 to May 12, 1913, Experiments 9 to 15 :—

Date.	Expt.	L.	D.	L.
1913.				
May 3	9	170	138	149
„ 5	10	110	100	104
„ 6	11	134	107	134
„ 7	12	144	121	132
„ 8	13	113	109	103
„ 10	14	70	65	67
„ 12	15	77	60	67
	Sum	818	700	756
	Or as	117 :	100 :	108

Or taking the average of the two *light* readings, we have—
L/D = 113/100

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* 'Plant Physiology,' by W. F. Ganong, 1908. The instrument is supplied by Messrs. Bausch and Lomb.

The following experiments were made in an improvised laboratory having a bright north light; but the dark room was not perfect and the plants could not be exposed to absolute darkness. The potometer used was of the Kohl type, having a horizontal tube 0·95 mm. internal diameter. The march of the meniscus was generally timed over half a centimetre. The meniscus is brought back to zero by turning a tap and allowing water to enter, as in the instrument designed by Prof. Ganong.

The rate of transpiration is corrected for ψ (relative humidity).

Experiment 16.—May 7, 1913. *P. laurocerasus.*

May 6.—Branch, with seven last year's leaves, gathered at night.

May 7, 10 A.M.—Finished vaselining and making incisions in the leaves. Fresh surface made to branch; apparatus at an east window; dull morning. ψ between 65 and 82 per cent. T 12·7–16·8° C.

For each period, light or dark, the average rate of transpiration is given.

Time.	Rate.	Time.	Rate.
	Light.	12.18 P.M.	Dark.
10.20 A.M.	302	1.47 ,,	187
10.40 ,,		1.51 ,,	
10.47 ,,	Dark.	1.52 ,,	Light.
11.10 ,,	229	2.16 ,,	255
11.18 ,,		2.22 ,,	
11.19 ,,	Light.	6.53 ,,	195
11.50 ,,	277	6.55 ,,	dull light.
12.9 P.M.			

Omitting the last reading (as giving the effect of dull light), we have the average rates: Light = 278, Dark 208, or L/D = 134/100.

Experiment 17.—May 12, 1913. *P. laurocerasus.*

9.45 A.M.—Cut fresh surface to a branch which had been in water since May 10. Dull sky. ψ 80–88 per cent. T 12·6–13·8° C. The average rate of transpiration for the light and dark periods is given as before:—

Time.	Rate.	Time.	Rate.
	Light.	2.36 P.M.	Dark.
10.41 A.M.	179	3.52 ,,	114
10.56 ,,		3.55 ,,	
10.57 ,,	Dark.	3.56 ,,	Light.
11.45 ,,	129	6.9 ,,	129
11.54 ,,		6.13 ,,	
11.56 ,,	Light.		
1.1 P.M.	172		
2.34 ,,			

The average of the three light readings is 160, of the two dark ones 122 or L/D = 160/122 = 131/100.

If the L reading for 6.9–6.13 P.M. is omitted, which is fairer owing to the fading of the light, we have L/D = 176/122 = 144/100.

Experiment 18.—May 14, 1913. *P. laurocerasus.*

10.20 A.M.—Branch, bearing last year's leaves, vaselined and fitted to potometer.

11.2 A.M.—Incisions made in leaves. North window, fair light. ψ 71–78 per cent. T 15·2–15·9° C.

Time.	Rate.	Average.	Corrected for ψ.
	Light.		
11.45 A.M.	223	228	228
11.46 „	228		
11.48 „	231		
11.52 „	231		
11.53 „	Dark.		
12.19 P.M.	184	182	208
12.25 „	180		
12.28 „	Light, fairly bright.		
4.44 „	202	196	190
4.46 „	191		
4.51 „	194		

The average of the two L periods is 209, which is practically equal to the transpiration in the D period. This result is not explicable, as the light was good at 4.44. Nor was there any evidence of the wood-vessels being blocked, as sometimes occurs in potometer experiments.

Average: L/D = 209/208 = 100·5/100·0.

Experiment 19.—May 17, 1913. *P. laurocerasus.*

Fresh surfaces cut to the branch on May 15, May 16, and May 17.

9.30 A.M.—ψ between 76 and 82 per cent. T 13·7–14·4° C.

Time.		Time.		Time.	
10.39 A.M. 10.42 „	Light. 168	*Circa* 10.45 A.M. 11.19 „ 11.30 „	Dark. 105	11.34 A.M. 2.0 P.M. 2.20 „	Light. 221

The average of the two light periods is 194. L/D = 194/105 = 185/100.

The following experiments were made by Miss Pertz on *P. laurocerasus* growing at the Botany School, Cambridge:—Shoots bearing five to seven leaves of the current year were cut under water, and fitted to the Bausch and Lomb (Prof. Ganong's) potometer. The leaves were vaselined and cut as usual, the incisions being usually four in number. The surface of the branch was always carefully greased.

The first column gives times of observation. The second the number of seconds occupied by the absorption of one degree of the potometer, *i.e.* of 0·01 c.c. The third column (R = rate) gives the reciprocals of the time readings (Column 2) multiplied by 10,000. Thus the hourly rate of absorption (R) is expressed in units of 0·0036 c.c. The fourth column gives the rate R corrected for ψ.

Experiment 20.—June 9, 1913. *P. laurocerasus.*

Seven leaves. Relative humidity (ψ) varying between 58 and 63. Temperature between 16·5 and 18·3.

Time.	Time in seconds.	R.	Corrected.
10.14 A.M.	Leaves greased. Cut: placed at east window.		
10.18 "	96·0	104·0	108·0 (bright day)
10.26 "	102·0	98·0	98·0
10.35 "	105·0	95·2	102·0
10.52 "	92·5	108·0	115·0
11.1 "	100·0	100·0	100·0
11.11 "	107·0	93·5	93·4
11.16 "	107·0	93·5	93·4
11.18 "	Placed in dark room.		
11.30 "	128·0	78·1	85·4
11.42 "	141·0	70·9	77·5
11.55 "	160·0	62·5	68·3
12.3 P.M.	161·0	62·1	70·0
12.15 "	175·0	57·1	62·4
12.20 "	Replaced at east window, rather dull.		
12.27 "	135·0	74·0	76·0
12.37 "	126·0	79·4	81·0
12.44 "	110·0	90·9	94·9

If we take the last (corrected) reading in each period we get—

	Light.	Dark.	Light.
	93·4	62·4	94·9
or	150	100	152

Average: L/D = 151/100.

The rest of the experiments are given in abbreviated form, *i.e.*, merely the rate R corrected for difference in ψ.

Experiment 21.—June 6, 1913. *P. laurocerasus.*

9.30 A.M.—Cut a shoot with nine leaves of current year and fitted to potometer after vaselining and cutting. At east window. Dull day. ψ 59–62 per cent.

Time.	R.	Time.	R.
10.34 A.M.	61·0		
10.37 "	98·5	11.42 A.M.	In dark room.
10.52 "	81·3	11.48 "	41·9
	(lighter)	12.3 P.M.	44·8
11.15 "	69·0	12.20 "	37·7
11.40 "	54·3	12.38 "	29·3

Result: L/D = 185/100.

Experiment 22.—June 7, 1913. *P. laurocerasus.*

10 A.M.—Young shoots, six leaves. At east window. Dull day. ψ 62–74 per cent.

Time.	R.	Time.	R.
10.15 A.M.	49·5		Placed in dark room.
10.25 "	48·0	11.39 A.M.	49·8
10.51 "	75·0	11.52 "	48·0
11.7 "	66·7	12.12 P.M.	40·1
11.22 "	59·4	12.24 "	38·3
11.30 "	58·8		

Result: L/D = 154/100.

Experiment 23.—June 12, 1913. *P. laurocerasus.*

11.50 A.M.—Young shoot, seven leaves, vaselined but no incisions made. ψ 52–63 per cent. T 17·9–20·3.

Time.	R.	Time.	R.
June 13, 1913.		11.7 A.M.	107·0
9.30 A.M.	Fresh surface to branch	11.13 "	97·4
	cut under water:	11.25 "	88·7
9.50 "	25·8	11.39 "	87·8
10.0 "	24·3	11.48 "	89·2
10.1 "	Cut leaves, *i.e.* usual	11.54 "	89·9
	incisions made.	11.55 "	Replaced at east window.
10.6 "	69·6	12.2 P.M.	85·5
10.10 "	85·5	12.12 "	99·8
10.36 "	109·0	12.29 "	96·9
10.42 "	113·6	12.38 "	105·0
10.47 "	113·6	12.47 "	105·0
10.55 "	117·6	12.50 "	108·0
10.56 "	Placed in dark room.		

Result: Light 131, Dark 100, Light 120. Average: L/D = 126/100.

Experiment 24.—June 13, 1913. *P. laurocerasus.*

June 13, 1913.—A shoot with nine leaves, none very young; vaselined June 14, 10.5 A.M. At east window. ψ 63–61 per cent. T 18·4–20° C.

Time.	R.	Time.	R.
10.19 A.M.	23·9	11.32 A.M.	128
10.24 ,,	26·5	11.38 ,,	131
10.26 ,,	Cut up leaves.	11.47 ,,	126
10.28 ,,	95·2	11.56 ,,	129
10.34 ,,	126·6	12.11 P.M.	118
10.37 ,,	140	12.12 ,,	Replaced at east window.
10.50 ,,	150	12.18 ,,	128
11.3 ,,	160	12.23 ,,	137
11.10 ,,	154	12.30 ,,	138
11.15 ,,	156	12.36 ,,	134
11.16 ,,	Placed in dark room.	12.52 ,,	140
11.23 ,,	144		
Result :—Light 132, Dark 100, Light 119. Average: L/D = 126/100.			

Experiment 25.—June 16, 1913. *P. laurocerasus.*

Shoot with seven leaves, none very young. Vaselined and placed at east window.

Time.	R.	Time.	R.
June 17, 1913.	Cut fresh surface to branch under water	10.36 A.M.	102·0
		10.45 ,,	105·0
9.35 A.M.	Placed at east window.	11.4 ,,	105·0
	ψ 56–66 per cent.	11.5 ,,	Placed in dark room.
	T 21·9–25·8	11.21 ,,	75·3
9.59 ,,	19·8	11.30 ,,	76·1
10.0 ,,	Cut up the leaves	11.42 ,,	78·6
10.4 ,,	67·1	11.50 ,,	76·7
10.10 ,,	95·5	11.52 ,,	Replaced at east window.
10.14 ,,	105·0	12.23 P.M.	106·0
10.23 ,,	109·3	12.33 ,,	106·0
10.30 ,,	103·0		
Result: Light 138, Dark 100, Light 137. Average: L/D = 138/100.			

Weighing Experiments (Laurel).

In a few experiments the transpiration was estimated by the loss of weight of a cut branch (laurel) in a bottle of water covered with a layer of olive oil. The branches had each six leaves, which were carefully vaselined and cut in the usual way. The stem and all buds also vaselined with care.

The experiments took place in the laboratory above referred to, in which darkness was not absolute.

The specimens weighed from 100 to 130 grm., and were only weighed to within 5 mgrm.

Experiment 26.—May 10, 1913. *P. laurocerasus.*

8.52 A.M.—At bright north light. ψ 71–88 per cent. T 11–13·6° C.

Time.		Loss.	
		Per hour.	Corrected for ψ.
		grm.	
9.37 A.M. 10.21 "	Light	0·252	219
10.32 " 11.21 "	Dark	0·114	127
11.21 " 12.3 P.M.	Light	0·180	167
12.3 " 1.3 "	Dark	0·107	122
1.3 " 1.59 "	Light	0·244	217 (sky very bright.)
2.0 " 3.3 "	Dark	0·113	129
Result, average L/D = 201/126 = 159/100.			

Experiment 27.—May 22, 1913. *P. laurocerasus.*

Leaves vaselined, but not incised until May 23.

May 23.—Light clouds. ψ 75–86 per cent. T 14·2–18·2° C.

Time.		Loss	
		Per hour.	Corrected for ψ.
		grm.	
9.44 A.M. 10.43 "	Light	0·249	271
10.47 " 11.52 "	Dark	0·134	167
11.52 " 12.44 P.M.	Light	0·294	254
12.44 " 1.44 "	Dark	0·250	208
1.44 " 2.51 "	Light	0·242	242
2.52 " 4.15 "	Dark	0·236	197
Average: L/D = 256/191 = 134/100.			

Experiment 28.—May 24, 1913. *P. laurocerasus.* ψ 77–88 per cent.
T 15·1–18·5° C.

Time.		Loss.	
		Per hour.	Corrected for ψ.
		grm.	
9.34 A.M. 10.27 „	Light.	0·328	290
10.27 „ 11.44 „	Dark.	0·129	153
11.44 „ 3.59 „	Light.	0·321	273
3.59 „ 5.0 „	Dark.	0·195	163
5.0 „ 6.48 „	Light.	0·217	217

Average : L/D = 245/158 = 155/100.

Or, omitting the last L period : L/D = 325/162, or 201/100.

Experiment 29.—May 25. *P. laurocerasus.* ψ 76–83 per cent. T 17·8–20·5.

Time.		Rate.	Corrected for ψ.
9.26 A.M. 10.38 „	Light	231	231
10.38 „ 11.44 „	Dark	155	162
11.44 „ 12.51 „	Light	264	264
12.51 „ 4.10 „	Light	257	236

Average : L/D = 244/162 = 151/100.

The results of the series of four weighings are :—

L/D = 159/100 ; 134/100 ; 201/100 ; 151/100. Average L/D = 161/100.

Experiments on Ivy (Hedera helix).—*July,* 1913.

In the following experiments by Miss Pertz the specimens were cut at night and placed in water, and on the following morning a fresh surface was cut under water.

The leaves and stems were then carefully vaselined and four incisions per leaf were made.

In all cases the transpiration rate is corrected for ψ.

Experiment 30.—July 1, 1913. Ivy.

10.20 A.M.—Shoot with seven leaves vaselined, no incisions made.

July 2, 9.30 A.M.—In potometer at east window. Dull. During the day ψ 60–70 per cent. T 18–19·6° C.

Time.	R.	Average.	Time.	R.	Average.
9.38 A.M.	27·8		10.43 A.M.	77·0	
9.41 ,,	27·8		10.53 ,,	71·9	
9.43 ,,	Cut up leaves with scalpel,		11.4 ,,	78·2	
	4 cuts each leaf.		11.11 ,,	74·6	76
9.47 ,,	75·8		11.18 ,,	76·9	
9.51 ,,	82·0		11.19 ,,	Replaced at east window.	
10.0 ,,	96·2		11.22 ,,	75·8	
10.17 ,,	91·3		11.27 ,,	82·0	
10.26 ,,	112·0		12.43 P.M.	91·0	
10.32 ,,	124·0	115	12.46 ,,	91·0	95
10.35 ,,	106·0		12.52 ,,	99·0	
10.35 ,,	Placed in dark room.				

	L.	D.	L.
Result	115	76	95
or	151	100	125

Average : L/D = 138/100.

Experiment 31.—July 4, 1913. Ivy.

11 A.M.—Shoot, 12 leaves, cut and vaselined.

July 5, 10 A.M.—In potometer at east window. ψ during day 63–68 per cent. T 16·7–18·4° C.

Time.	R.	Average.	Time.	R.	Average.
10.15 A.M.	32·0		11.17 A.M.	121·0	
10.19 ,,	29·7		11.40 ,,	106·0	
10.22 ,,	Cut up leaves with scalpel,		11.51 ,,	102·0	103
	4 cuts each leaf		11.59 ,,	104·0	
10.25 ,,	108·0		12.0 NOON	Replaced at east window.	
10.31 ,,	122·0		12.8 P.M.	115·0	
10.41 ,,	126·0		12.18 ,,	123·0	
10.52 ,,	137·0		12.33 ,,	125·0	
11.1 ,,	139·0		12.42 ,,	127·0	
11.9 ,,	147·0	147	12.47 ,,	123·0	
11.11 ,,	147·0		12.51 ,,	131·0	133
11.12 ,,	Placed in dark room		12.53 ,,	134·0	

	L.	D.	L.
Result	147	103	133
or	143	100	129

Average : L/D = 136/100.

Experiment 32.—July 7, 1913. Ivy.

11 A.M.—Shoot cut, 10 leaves (current year) vaselined.

July 8, 9.55 A.M.—In potometer at east window. ψ during day 54–64 per cent. T 15·2–17·1° C.

Time.	R.	Average.	Time.	R.	Average.
10.5 A.M.	21·5		11.2 A.M.	115·0	
10.9 ,,	20·7		11.18 ,,	111·0	
10.11 ,,	Cut up leaves, 4 cuts per leaf.		11.25 ,,	118·0	
			11.31 ,,	120·0	119
10.13 ,,	85·5		11.35 ,,	118·0	
10.17 ,,	100·0		11.35 ,,	Replaced at east window.	
10.24 ,,	109·0		11.46 ,,	133·0	
10.32 ,,	125·0		11.52 ,,	133·0	
10.39 ,,	127·0		12.2 P.M.	138·0	
10.47 ,,	125·0	129	12.12 ,,	140·0	
10.49 ,,	132·0		12.19 ,,	140·0	139
10.50 ,,	Placed in dark room.		12.24 ,,	138·0	

	L.	D.	L.	
Result	129	119	139	Average : L/D = 113/100.
or	108	100	117	

Experiment 33.—July 9, 1913. Ivy.

10 A.M.—Shoot having 13 leaves of current year, cut and vaselined.

July 10.—In potometer at east window. ψ during day 65–73 per cent. T 16·7–19·2° C.

Time.	R.	Average.	Time.	R.	Average.
10.11 A.M.	Cut up leaves, 4 cuts each leaf.		11.51 A.M.	120	121
			11.56 ,,	121	
10.17 ,,	96		11.57 ,,	Replaced at east window.	
10.26 ,,	110		12.4 P.M.	150	
10.38 ,,	130		12.9 ,,	154	
10.47 ,,	147		12.16 ,,	157	
10.51 ,,	143		12.22 ,,	152	
10.57 ,,	135	140	12.27 ,,	154	
10.59 ,,	145		12.32 ,,	151	
11.0 ,,	Placed in dark room.		12.35 ,,	159	
11.11 ,,	112		12.38 ,,	149	153
11.49 ,,	123		12.39 ,,	157	

	L.	D.	L.	
Result	140	121	153	Average : L/D = 121/100.
or	116	100	126	

Experiment 34.—July 10, 1913. Ivy.

Shoot having nine leaves, six being of current year, vaselined.

July 11, 10.20 A.M.—In potometer at east window. ψ during day 66–69 per cent. T 17–18·9° C. Dull morning.

Time.	R.	Average.	Time.	R.	Average.
10.29 A.M.	22·6		11.52 A.M.	71·0	
10.31 ,,	Cut up leaves, 4 cuts each leaf.		12.6 P.M.	75·0	75
			12.18 ,,	74·0	
10.34 ,,	88·0		12.20 ,,	Replaced at east window.	
10.40 ,,	83·0		12.26 ,,	80·0	
10.49 ,,	85·0		12.32 ,,	91·0	
11.7 ,,	98·0		12.44 ,,	89·0	
11.22 ,,	93·0	95	12.59 ,,	95·0	
11.25 ,,	97·0		1.7 ,,	94·0	
11.26 ,,	Placed in dark room.		1.12 ,,	103·0	101
11.34 ,,	82·0		1.18 ,,	98·0	
11.40 ,,	76·0				

	L.	D.	L.	
Result	95	75	101	Average : L/D = 131/100.
or	127	100	135	

Experiment 35.—June 22, 1913. Ivy.

The following experiment may be placed with the above, although transpiration was estimated by weighing instead of with the potometer. The method was the same as that described for laurel.

June 21, 4.30 P.M.—Branch cut under water and placed in water covered with a layer of oil. The lower surfaces of the leaves (of the current year) carefully vaselined, together with the stem.

June 22, 9.40 A.M.—Four or five incisions made per leaf. Placed in north window; the sky fairly bright. ψ from 72 to 80 per cent. T 16·6–19·2° C.

Time.		Rate corrected for ψ.	Time.		Rate corrected to ψ.
10.18 A.M. 11.20 ,,	Light	295	12.34 P.M. 1.37 ,,	Light	361
11.20 ,, 12.34 P.M.	Dark	187	1.37 ,, 2.41 ,,	Dark	190

The average transpiration L/D = 328/189
or L/D = 174/100

The average of the L/D results for ivy, viz., 138, 136, 113, 121, 131, 174/100, is 136/100.

Results.

§ 1. The method employed was to close the stomata by carefully rubbing the stomatal surface with cocoa-fat or vaseline, the intercellular spaces being afterwards put in communication with the outer air by means of incisions.

In the case of leaves not thus treated, it is well known that the closure of the stomata in darkness greatly diminishes the evaporating surface and *vice versâ*. In my method the evaporating surface is a constant.

§ 2. The following tables give the comparative effects of diffused daylight and darkness on the transpiration of *P. laurocerasus* and *Hedera helix* treated as in § 1.

P. laurocerasus.

Date.	Light.	Dark.	Date.	Light.	Dark.
1911.			1913.		
April 11	105	100	May 14	101	100
,, 19	100	100	,, 17	185	100
,, 20	148	100	,, 22	134	100
,, 22	123	100	,, 24	201	100
1913.			,, 25	151	100
April 21	113*	100	June 6	185	100
May 3			,, 7	154	100
,, 3	116	100	,, 9	151	100
,, 5	107	100	,, 12	126	100
,, 6	125	100	,, 13	126	100
,, 7	114	100	,, 16	138	100
,, 7	134	100	1911.		
,, 8	100	100	Nov. 15	125	100
,, 10	105	100	,, 17	114	100
,, 10	159	100	1909.		
,, 12	120	100	Dec. 9	119	100
,, 12	144	100			

Average L/D : 131 ·7/100, in round numbers 132/100 or 32 per cent.

* The average of eight experiments, see p. 286.

Ivy (*Hedera*).

Date.	Light.	Dark.	Date.	Light.	Dark.
June 22, 1913	174	100	July 7, 1913	113	100
July 1, ,,	138	100	,, 9, ,,	121	100
,, 4, ,,	136	100	,, 10, ,,	131	100

Average L/D : 136/100 or 36 per cent.

§ 3. The tables given under § 2 show a remarkable degree of variability: the extreme cases are: April 19, 1911, when the result was *nil*, and May 24, 1913, when the transpiration in light was double that in darkness. The average ratio for transpiration in light and darkness is: ivy, 136/100; laurel, 132/100. But between May 14 and June 16 the laurel gives an average 150/100, and, speaking generally, it cannot be doubted that the laurel reacts to illumination more in early summer than in spring. The winter experiments are not sufficiently numerous to justify any comparison with those obtained in summer.

It is at present impossible to form any conclusion as to the cause of the increased reaction in June. I have no evidence as to whether the increased permeability to water is a periodic effect, or connected with the age of the leaf, or with the brightness of the summer sky, as compared with illumination earlier in the year.

§ 4. With regard to the main fact that transpiration is increased by light or diminished by darkness, we may either accept the view of Wiesner,* viz., that in light the chloroplasts are warmed by the absorption of radiant energy, or we may believe that light produces an increased permeability of the plasmic membrane to water, a point of view to which the interesting work of Lepeschkin and Tröndle† on the increased permeability to dissolved substances produced by illumination may possibly give some support. Or we may combine Wiesner's theory with those of the other writers.

It is a pleasure to express my thanks to Miss D. F. M. Pertz for the valuable aid she has given me throughout the research.

* Wiesner, 'Sitzb. d. k. Akad. Wiss.,' 1877, vol. 74, p. 477.

† Lepeschkin, 'Ber. d. Bot. Ges.,' xxvi, *a*; Tröndle, *ibid.* xxvii.

The Chemical Interpretation of some Mendelian Factors for Flower-Colour.

By M. Wheldale, Fellow of Newnham College, Cambridge, and
H. Ll. Bassett, Trinity Hall, Cambridge.

(Communicated by W. Bateson, F.R.S. Received November 21, 1913,—Read January 22, 1914.)

(From the Laboratory of the John Innes Horticultural Institution, Merton, Surrey, and the Balfour Laboratory, Cambridge.)

The inheritance of flower-colour in *Antirrhinum majus* has been worked out by one of us* and also by Baur.† Investigation has shown that the flower-colour of the type in *Antirrhinum* is due to the presence of at least six factors and that these, in various combinations, produce a series of colour-varieties. Full accounts of the factors have been given in the papers cited, but for convenience of reference four are mentioned again here, *i.e.*:—

Y. A factor representing the power to form ivory pigment in the tube, accompanied by yellow pigment in the lips.
I. A factor representing the power to form ivory pigment in the lips.
R. A factor representing the power to form red pigment in the flower.
B. A factor representing the power to convert red into magenta pigment.

The factorial constitution of the type and varieties can be expressed as follows:—

YY(y)iirrB(b)B(b)	Yellow.
YY(y)II(i)rrB(b)B(b)	Ivory.
YY(y)iiRR(r)bb	Bronze.
YY(y)II(i)RR(r)bb	Red.
YY(y)iiRR(r)BB(b)	Crimson.
YY(y)II(i)RR(r)BB(b)	Magenta.
yyI(i)I(i)R(r)R(r)B(b)B(b)	White.

In 1909 a series of researches was commenced with a view to the interpretation of the above factors in terms of chemical substances, or possibly enzymes. Since some of the pigments involved (red, magenta) belong to the group of so-called anthocyanins, *i.e.* soluble red, purple and blue pigments of plants, general investigations were at first made by one of us* on anthocyanin pigments. As a result of qualitative reactions, in conjunction with evidence from cross-breeding, it was suggested that anthocyanins, as a group, are oxidised products of the natural yellow colouring matters, the flavones and xanthones. At the same time it was pointed out that a number of the latter substances had been isolated by Perkin and others† from various plants and several had been shown to be widely distributed. The existence of many flavones and flavone derivatives was mentioned and attention was drawn to the fact that, as a group, they have similar properties but differ among themselves in the number and position of their hydroxyl groups and in other points. It was further suggested that the oxidised products (anthocyanins) might, in a similar way, form a group of closely related substances, differing individually according to the flavone from which each had been derived.

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* Wheldale, M., "The Inheritance of Flower-colour in *Antirrhinum majus*," 'Roy. Soc. Proc.,' 1907, B, vol. 79, p. 288; "Further Observations on the Inheritance of Flower-colour in *Antirrhinum majus*," 'Rep. Evol. Com. Roy. Soc.,' V, 1909, p. 1.

† Baur, E., "Einige Ergebnisse der experimentellen Vererbungslehre," 'Beihefte zur Med. Klinik,' Berlin, 1908, Heft 10, p. 265; "Vererbungs- und Bastardierungsversuche mit *Antirrhinum*," 'Zs. indukt. Abstammungslehre,' Berlin, 1910, vol. 3, p. 34.

In view of evidence collected from various sources, it was again suggested by one of us,‡ that since the flavones are known to be present in many cases as glucosides in the plant, the reactions involved in the formation of anthocyanin might be stated in very general terms as follows:—

$$\text{Glucoside} + \text{water} \rightleftarrows \text{flavone} + \text{sugar},$$

$$x\,(\text{flavone}) + \text{oxygen} \rightarrow \text{anthocyanin},$$

and also that, in addition to oxidation, there might be condensation of the flavone molecules. It was likewise stated that the first reaction might be controlled by a glucoside-splitting enzyme and the second, if due to oxidation, by an oxydase.

Subsequent work has strengthened the view that anthocyanins are, in all probability, derivatives of the flavones, though we ourselves have no further evidence as to the actual nature of the reactions involved in their formation.

Since we find little reliance can be placed on results given by crude water or alcoholic extracts from flowers, in all later investigations an attempt has been made to deal with the isolated and purified pigments. In a paper by one of us,§ the methods of preparation and purification of the crude pigment

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[*] Wheldale, M., "The Colours and Pigments of Flowers with special Reference to Genetics," 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 44; "On the Nature of Anthocyanin," 'Phil. Soc. Proc.,' Cambridge, 1909, vol. 15, p. 137.

[†] Perkin, A. G., various papers in 'Chem. Soc. Trans.,' from 1895 to 1904.

[‡] Wheldale, M., "On the Formation of Anthocyanin," 'Journ. Genetics,' 1911, vol. 1, p. 131.

[§] Wheldale, M., "The Flower Pigments of *Antirrhinum majus.* I.—Method of Preparation," 'Biochem. Journ.,' 1913, vol. 7, p. 87.

have been described. In a more recent paper by both authors,* an account has been given of the identification of the ivory pigment of *Antirrhinum* with apigenin, a flavone of known constitution, isolated by Perkin† from apiin, a glucoside occurring in the parsley, *Apium petroselinum*. Apigenin is a very pale yellow crystalline substance, readily soluble in hot alcohol, slightly so in ether and almost insoluble in water. Melting point, 347° C. In the *Antirrhinum* plant, apigenin undoubtedly exists as a glucoside, in which state it is more soluble than after hydrolysis.

Attention has been given subsequently to the yellow pigment and the results are included in the present paper. The crude pigment prepared from yellow flowers was extracted with ether by methods described in previous papers. The ether extract contains apigenin from the tube and inner tissues of the corolla, and yellow pigment from the epidermis of the lips, including the patch on the palate. It was at first thought that the yellow pigments in the epidermis of the lips and in the patch on the palate might be identical. After removing the bulk of the apigenin from the ether extract by crystallisation from alcohol, the remaining yellow pigment, which is very soluble in alcohol, gave, on fractional crystallisation from dilute alcohol, products of which the melting points varied from about 250° to 338° C.

The wide range of the melting points, combined with certain qualitative reactions of these extracts, led to the conclusion that the palate contained the lip pigment mixed with other pigments, or even other pigments without the lip pigment. Since, however, the patch on the palate is common to all varieties (except white), the factorial difference between ivory and yellow is only concerned with the yellow lip pigment. Hence, in order to simplify the problem, the pigments of the palate have been disregarded for the time being, and investigations have been limited to crude material (unfortunately prepared only in small quantity) from the upper lips of the yellow variety. In this product, it seemed more likely that there would only be two pigments present to any extent.

Even the more simple mixture presented very great difficulties in the separation of yellow from ivory, both pigments having almost the same solubilities in all solvents used. Such separation as was possible by means of different solubilities gave products which indicated by their melting points, 300–328° C., the presence of luteolin, this substance being the only known flavone melting above 300° C. and having at the same time the solubilities and properties of the yellow pigment.

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* Wheldale, M., and Bassett, H. Ll., "The Flower Pigments of *Antirrhinum majus*. II.—The Pale Yellow or Ivory Pigment," 'Biochem. Journ.,' 1913, vol. 7, p. 441.

† Perkin, A. G., "Apiin and Apigenin," 'Chem. Soc. Journ., Trans.,' 1897, vol. 71, p. 805; 1900, vol. 77, p. 416.

Proceeding on the assumption that the yellow pigment might be luteolin, a fairly satisfactory separation was brought about by hydrobromic acid, which, according to Perkin,* forms, in glacial acetic acid, a compound with luteolin but not with apigenin. The luteolin hydrobromide remains in solution unless excess of hydrobromic acid is added, when it separates out in ochre-coloured crystals which are decomposed by water into luteolin and hydrobromic acid. The method of procedure in our case was as follows: The ether extract containing the mixed pigments was ground into a thin paste with glacial acetic acid, heated to boiling, and hydrobromic acid added, but not in excess. On cooling, the bulk of the apigenin separated out, while the yellow pigment remained in solution. The apigenin was filtered off, and on addition of much water to the filtrate the yellow pigment separated out and was also filtered off. A repetition of this process ensures greater purity of the yellow. After drying, the yellow was further purified by extraction with ether.

The pigment prepared in this way, except for its melting point, which varied from 310° to 328° C., resembled luteolin in properties. According to Perkin,† luteolin is a bright yellow crystalline substance, readily soluble in alcohol, fairly soluble in ether, and very slightly soluble in water, even when hot. With ferric chloride solution luteolin gives at first a green, later a red-brown, coloration. The melting point of luteolin was for many years given as "above 320° C." More recently Perkin has obtained luteolin by two different methods of purification, giving, in one case, a product melting at 327–329° C., in the other, at 323–326° C.

Luteolin occurs in *Genista tinctoria*‡ and in leaves of *Digitalis*§ and also, together with small quantities of apigenin, in *Reseda luteola*.||

The structural formulæ of luteolin and apigenin are as follows:—

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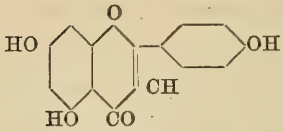
* Perkin, A. G., "Luteolin.—Part I," 'Chem. Soc. Journ., Trans.,' 1896, vol. 69, p. 206.

† Perkin, A. G., "Luteolin.—Part I," 'Chem. Soc. Journ., Trans.,' 1896, vol. 69, p. 206; "Luteolin.—Part II," 'Chem. Soc. Journ., Trans.,' 1896, vol. 69, p. 799; Perkin, A. G., and Horsfall, L. H., "Luteolin.—Part III," 'Chem. Soc. Journ., Trans.,' 1903, vol. 77, p. 1314.

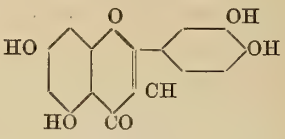
‡ Perkin, A. G., and Newbury, F. G. "The Colouring Matters contained in Dyer's Broom (*Genista tinctoria*) and Heather (*Calluna vulgaris*)," 'Chem. Soc. Journ., Trans.,' 1899, vol. 75, p. 830.

§ Fleischer, F., "Digitoflavon, ein neuer Körper aus der *Digitalis purpurea*," 'Ber. D. Chem. Ges.,' 1899, vol. 32, p. 1184; Killiani, H., u. Mayer, O., "Ueber die Identität von Digitoflavon und Luteolin," 'Ber. D. Chem. Ges.,' 1901, vol. 34, p. 3577.

|| Perkin, *loc. cit.*



Apigenin.



Luteolin.

As pointed out by Smiles,[*] the more intensely coloured flavones contain two hydroxyl groups in the ortho position with respect to one another, whereas the arrangement in apigenin is not so productive of colour.

The close connection between the structure of the two substances, and the fact of their occurrence together in *Reseda luteola*, also favour the assumption that the yellow *Antirrhinum* pigment is luteolin. The presence of luteolin in the allied genus *Digitalis* is also of interest.

In order to corroborate this suggestion, attempts were made to form both the acetyl and benzoyl derivatives of the yellow pigment. The attempts failed, owing partly to the small amount of pigment available, and partly to the following difficulties. In the case of the acetyl derivative, the method of dissolving the pigment in caustic soda or pyridine and adding acetyl chloride apparently failed to acetylate the pigment completely. The method employed by Perkin and others of boiling the pigment with acetic anhydride and anhydrous sodium acetate was not found satisfactory when dealing with such small amounts of substance, since there were produced simultaneously brown decomposition products, from which it was impossible to isolate the derivative. In attempts to benzoylate the yellow pigment by the Schotten-Baumann method, the same difficulties arose, together with a further one, namely, the fact that the melting point, 201° C., of the benzoyl derivative of luteolin is only about 10° lower than that, 210–212° C., of the benzoyl derivative of apigenin; hence the possibility that the small amount of product formed might be impure apigenin derivative produced from apigenin retained in the luteolin used. It has been shown by Perkin[†] that in the Schotten-Baumann method, under certain conditions, a tribenzoyl, instead of a tetrabenzoyl, derivative may be formed.

Finally, attempts were made to form the benzol sulphonyl derivative described by Fleischer[‡] as obtained from digitoflavone, the latter substance being extracted from *Digitalis* leaves and subsequently shown to be identical with luteolin. Fleischer's benzol sulphonyl derivative was obtained by treating the digitoflavone, in caustic soda solution, with benzol sulphochloride and crystallising the product from a mixture of chloroform and ether. Melting point, 189° C.

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[*] Smiles, S., 'The Relations between Chemical Constitution and some Physical Properties,' London, 1910.

[†] Perkin, A. G., "Notes on Luteolin and Apigenin," 'Chem. Soc. Journ., Trans.,' 1902, vol. 81, p. 1174.

[‡] Fleischer, F., *loc. cit.*

By treating a specimen of yellow pigment, purified by means of the hydrobromide method and subsequent extraction with ether, in a similar way with benzol sulphochloride, an almost white product was obtained, which crystallised from a mixture of chloroform and ether and melted at 188–190° C.

By hydrolysing a small quantity of the benzol sulphonyl derivative with alcoholic soda for three hours, a sample of luteolin was obtained, melting at 324° C.

There is no doubt that the yellow *Antirrhinum* pigment is luteolin. The factorial difference between the yellow and ivory varieties can, therefore, be expressed as follows:—The ivory variety has the power to form apigenin throughout the tissues of the flower, whereas the yellow variety has the power to form luteolin, either in addition to, or more probably instead of, apigenin, in the upper epidermis of the lips. It appears most likely that the yellow variety has lost the power to form apigenin in the epidermis and produces luteolin instead, though there does not seem to be any particular reason why the power to form apigenin, instead of luteolin, should be a dominant character.* The different flavones synthesised in either case may be regarded rather as an expression of a fundamental difference in structure of the living molecule in the two varieties, affecting, perhaps, the production of different hydroxybenzoic acids, from which the flavones may be synthesised. Little can be gained at present by postulating the existence of a special organic catalyst or enzyme, representing the "I" factor, and concerned with the removal or addition of an hydroxyl group.

From the white variety no flavones could be extracted, and this is in accordance with Mendelian evidence. We must suppose, therefore, that either the substances from which the flavones are synthesised are absent, or the power of synthesis fails.

As regards the yellow patch on the palate, it appears likely that other flavones, having lower melting points and slightly deeper colour than luteolin, are present in this region.

It seems highly probable that the anthocyanin pigments are derived from the flavones by oxidation, or condensation, or both, though only accurate analyses of the pure pigments can ultimately decide this question. With regard to the suggestion made by one of us as to the mode of formation of anthocyanin from the flavone, *i.e.*, that the hydroxyls of the flavones may be protected by sugar, so to speak, and only after hydrolysis can changes take place at these points, there is no very definite evidence as to the number of sugar molecules attached to flavones in the plant. Careful isolation and analysis would be necessary to ascertain the actual condition in the living plant, owing to the great ease with which hydrolysis takes place after death.

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* There are probably very small quantities of other flavones in the lips of both yellow and ivory, but these do not affect the mass colour of the flowers.

Red and magenta anthocyanin have been obtained by us from *Antirrhinum* in a fairly pure state, and certain derivatives have been made. The fact that these, as well as the pigments, are practically amorphous indicates that they probably have very high molecular weights. The lack of melting points in the pigments supports this view.

In a recent paper Keeble, Armstrong, and Jones* bring forward an hypothesis to explain the loss of colour when coloured petals are treated with strong alcohol, and the subsequent restoration of colour when they are treated with water.

The phenomena recorded are as follows:—When coloured (anthocyanin) petals of Stocks (*Matthiola*) are placed in strong alcohol, some pigment passes into solution in the alcohol, which at first is coloured but fairly rapidly becomes colourless. The petals also become colourless though more slowly. When the colourless petals are taken out and placed in water the colour returns; in hot water the recovery is more rapid. When the extract is filtered from the petals and evaporated to dryness on a water-bath the colour returns to the residue. In addition we have noted that colour returns to the alcoholic filtrate on dilution with water, and this also happens even after evaporation to dryness and taking up again with alcohol.

The above phenomena are exhibited by most pigments of the anthocyanin class, and have been noted by various authors working on anthocyanin, among whom may be mentioned Hansen,† Molisch,‡ and Grafe.§

The hypothesis brought forward by Keeble, Armstrong, and Jones to explain these phenomena is the following:—The petals contain an oxydase and a reducing agent, which is probably not an enzyme. The oxydase is responsible for the production of anthocyanin from the chromogen, and the reducing agent reverses the reaction. With a decrease in amount of water in the cell the reducing agent becomes active and the oxydase inert, but with an increase in amount of water the oxydase becomes active and its effect is greater than that of the reducing agent. Hence, when petals are treated with strong alcohol the oxydases can no longer function, and the reducing agent is then able to reduce the anthocyanin to a colourless leuco-compound. On addition of water the oxydase again becomes active and re-oxidises the leuco-compound.

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* Keeble, F., Armstrong, E. F., and Jones, W. N., "The Formation of the Anthocyan Pigments of Plants. Part IV.—The Chromogens," 'Roy. Soc. Proc.,' 1913, B, vol. 86, p. 308.

† Hansen, A., 'Die Farbstoffe der Blüthen und Früchte,' Würzburg, 1884.

‡ Molisch, H. J., "Ueber amorphes und kristallisiertes Anthokyan," 'Bot. Zeit.,' Leipzig, 1905, vol. 63, p. 145.

§ Grafe, V., "Studien über das Anthokyan.—Mittheilung 3," 'Sitzb. Ak. Wiss. Wien,' 1911, vol. 120 (1), p. 765.

Such is the hypothesis, but we are not clear as to the explanation offered by the authors for the reappearance of colour in the alcoholic solution apart from the petals. Two alternatives offer themselves. First, that both oxydase and reducing agent are extracted by 95–99-per-cent. alcohol and are present in the alcoholic extract and that neither is affected by heating to 100° C.* (in spite of the fact that extraction by absolute alcohol and resistance to heat is not characteristic of oxydases), and that, although the authors quote experiments to prove that the oxydase can oxidise to some extent in 95-per-cent. alcohol, the reducing agent is more powerful in this medium. Or, that the reducing agent alone is extracted by alcohol and its influence is removed by evaporating the alcohol or by diluting, when re-oxidation occurs merely on exposure to air. If the latter be the case, the presence of the oxydase is superfluous to the recovery of colour in the petals themselves. We must also conclude that the reducing agent is very widely distributed, is unaffected by temperature of 100° C., and can only act in presence of alcohol.

To us the reduction and oxidation hypothesis appears directly opposed to essential experimental facts, although the original production of anthocyanins in the plant is, in all probability, either partly or wholly due to the action of an oxydase on a chromogen, most likely a flavone or xanthone.

In our experiments, various coloured petals of Stocks were used, and these were the flowers also used by Keeble, Armstrong, and Jones.

Experimentally we found that the same results are given both by the decolorised petals and by the alcoholic solution.

We find that if a little acid is added to absolute alcohol containing decolorised petals, the usual red colour reaction of acid with an anthocyanin is obtained both in the solution and in the petals. Moreover the same result is obtained equally well when dry hydrochloric acid gas or dry hydriodic acid gas is passed through the alcohol. Also, contrary to the observations of Keeble, Armstrong, and Jones, we find that prussic acid gas acts quite as well as any other acid, which would not be the case if an enzyme were responsible for the restoration of colour.

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* In a later paper (Jones, W. N., "The Formation of Anthocyan Pigments. Part V.—The Chromogens of White Flowers," 'Roy. Soc. Proc.,' 1913, B, vol. 86, p. 318) the author definitely states that oxydase is destroyed by boiling 50-per-cent. alcohol.

In any of these cases when the anthocyanin restored by acid is made alkaline, the greenish colour reaction of anthocyanin is obtained, showing that the restored colour is actually due to anthocyanin. The greenish reaction is also produced directly when a drop of a solution of caustic soda in absolute alcohol is added to the alcohol containing the decolorised petals.

If water is boiled to expel oxygen and carbon dioxide, and, while still hot, a stream of hydrogen is bubbled through it, this water, while the hydrogen is still passing, restores the colour to decolorised petals. In this case the medium is neutral.

It is not conceivable that oxidation can take place in all these experiments, particularly in that with dry hydriodic acid gas. Clearly also water is not necessary for the change, and another explanation for the restoration of colour must be sought.

Further, if reduction is the cause of decolorisation, the conditions in some of these experiments are exactly those most suited for the continued stable existence of the leuco-compounds, so that it would seem that this explanation must also be abandoned.

There may be a reducing agent present in the petals, but its reducing power cannot be responsible for the loss of colour in alcohol.

In support of their theory that reduction is the cause, Keeble, Armstrong, and Jones, in a later paper,* quote the fact that an extract from the petals is reduced to a colourless state by treatment with zinc dust and acid, and that the colour is restored by exposure to air. We would note in passing that this does not seem to be simply a reducing action, as we find that the restored colour is much fainter with acetic than with sulphuric acid. This observation has been made previously by Kastle,† who also does not consider it simply a reducing action. Untreated anthocyanin gives exactly the same colour with acetic as with sulphuric acid.

A point we wish to emphasize, however, is that we find the slightly acid solution to be easily decolorised by warming with a little hydrogen peroxide and colloidal platinum. The colourless oxidation product so formed is unstable, and the colour is restored if the solution is made more strongly acid.

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* Keeble, F., Armstrong, E. F., and Jones, W. N., "The Formation of Anthocyan Pigments in Plants.—Part VI," 'Roy. Soc. Proc.,' 1913, B, vol. 87, p. 113.

† Kastle, J. H., "A Method for the Determination of the Affinities of Acids Colorimetrically by Means of certain Vegetable Colouring Matters," 'Amer. Chem. Journ.,' 1905, vol. 33, p. 46.

Since anthocyanin can thus be decolorised by oxidation as well as by reduction, in each case giving a product in which the colour is easily restored, there is as much reason, on the evidence of these experiments, to postulate one process as the other for the cause of decolorisation by treatment with alcohol. As a matter of fact, the conditions in both experiments are so different from those obtaining when petals are treated with alcohol, that probably neither experiment has any real bearing on the question at all.

That an alternative to the reduction and oxidation hypothesis can be offered, is shown by the parallel series of changes produced by using phenolphthalein solution, made red by ammonia, as a pigment. This red solution is decolorised by alcohol and the colour restored by diluting largely with water or by addition of a drop of alkali. On evaporating the decolorised alcoholic solution to dryness, a red residue is obtained. As it happens, phenolphthalein is colourless with acids, while anthocyanin gives colour reactions with both acid and alkali. Apart from this accidental difference, the two cases are strikingly similar.

Without wishing to insist on the parallel too rigidly, it would seem that the two series of phenomena might well have similar explanations. The present authors tentatively offer two alternative suggestions without attempting to decide between them.

It may be that strong alcohol dehydrates the anthocyanin, giving a colourless compound, and that colour is restored by subsequent addition of two radicals, either H and OH, or some other pair, such as H and I. Such an effect might perhaps be accounted for by the production in anthocyanin of a lactone grouping. A somewhat similar explanation has been advanced to account for the phenolphthalein changes.*

Or, the loss of colour when the petals are treated with alcohol may be due to combination of the anthocyanin with alcohol to an unstable colourless compound, which is easily decomposed by various reagents. A similar explanation has been advanced by Hantzsch to account for the differently coloured solutions given by certain substances in different solvents.

A few minor points in connection with the above work may be considered.

First, Keeble, Armstrong, and Jones state that the restoration of colour to petals is accelerated by a drop or two of hydrogen peroxide either in acid or alkaline medium, and, further, that the reappearance of colour is not due to the acidity or alkalinity of the medium, because the original colour, purple, red or pink in differently colored petals, is first restored, and the acid or alkaline anthocyanin colour only appears later.

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* Meyer, R., u. Spengler, O., "Zur Constitution der Phtaleïnsalze," 'Ber. D. Chem. Ges.,' 1905, vol. 38, p. 1318.

Since we find that the return of colour in water is always accelerated by acid or alkali, we suggest that the acceleration by hydrogen peroxide is merely a function of the amount of acidity or alkalinity of the medium in which the hydrogen peroxide is dissolved. Moreover, although the exceedingly small amount of acid or alkali which at first diffuses into the petal from the very dilute solution may be sufficient to accelerate the actual return of colour, it is not sufficient to give the usual acid or alkaline coloration with the anthocyanin present. Further addition of the hydrogen peroxide solution would, and in fact does, bring about this result. In support of this, we observe that the extract, which at once comes into contact with the full amount of acid or alkali, immediately gives the acid or alkali colour, and not the original purple, pink, etc., of the petals.

To confirm this suggestion we carefully neutralised some laboratory hydrogen peroxide, which is, of course. always decidedly acid, and found that this neutral reagent actually retarded the recovery of colour as compared with control experiments on decolorised petals in cold, hot, or very faintly acidified water.

This result is not surprising in view of the decolorisation of petals by hydrogen peroxide and colloidal platinum, already described in this paper, and, we think, clearly demonstrates that the oxidising properties of hydrogen peroxide have nothing to do with the recovery of colour by the use of this reagent when it has not been neutralised.

Secondly, the same authors state that the purple coloration of a petal can be restored by re-oxidation in an acid medium. For this purpose purple petals of Stocks are incubated with 99-per-cent. alcohol with just enough citric acid to render the alcohol acid. The petals become almost decolorised, but retain a faint pink colour. When transferred to distilled water the pigment is reproduced in considerable quantity, at first red and then purple.

We should explain the phenomenon as follows: The purple pigment is rendered colourless by the alcohol, but, owing to the presence of a small quantity of citric acid (which is very slightly dissociated in alcohol), the colour does not entirely disappear, and the solution remains pink. Transference to water restores the colour, which is at first red, owing to the increased ionisation of the citric acid by the water that soaks into the petal. After a time the acid diffuses away into the surrounding water, leaving the liquid in the petals practically neutral, when the pigment becomes purple.

Finally Keeble, Armstrong, and Jones note that when the colour is restored to petals by immersion in water, and the colour is allowed to diffuse out of them, coloration is again restored by transferring them to hot water, and this process may be repeated two or three times.

They hold that the successive restorations of colour are due to fresh supplies of chromogen being produced by the plant under the influence of the hot water, and that each fresh amount is then oxidised to anthocyanin.

We suggest that these phenomena are explained by the fact that though a certain amount of pigment diffuses out into the water, a large proportion of that which was originally present is retained by the coagulated proteins of the petals, of course in the colourless state. It is the successive liberation of fractions of this retained pigment that accounts for the fresh production of colour in hot water, and not a new formation of chromogen.

On the Heat Production Associated with Muscular Work.[*]

By R. T. Glazebrook, M.A., F.R.S., and D. W. Dye, B.Sc.

(Received December 1, 1913,—Read January 22, 1914.)

On reading Prof. Macdonald's paper it appeared that it might be interesting to see if his results connecting the heat production and muscular work could be expressed graphically or by means of some simple formula. The tables in his paper give the heat production in calories per hour of a number of individuals when doing a carefully measured amount of mechanical work on a kind of treadmill or cycle. This amount of work is kept constant for each group of observations in the paper. Table I gives his average results.

Table I.

	Mechanical work.	Heat production.	
		From observation.	From formula.
Group A	13	182	179
B	19	199	202
C	43	297	296
D	56	346	347

On plotting these as is done in fig. 1, it is clear that the points lie very approximately on a straight line, and it is easily seen that the equation to this line may be written

$$H = 128 + \frac{W}{0{\cdot}256}; \qquad (1)$$

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[*] A Note on Prof. J. S. Macdonald's paper, *supra*, p. 96.

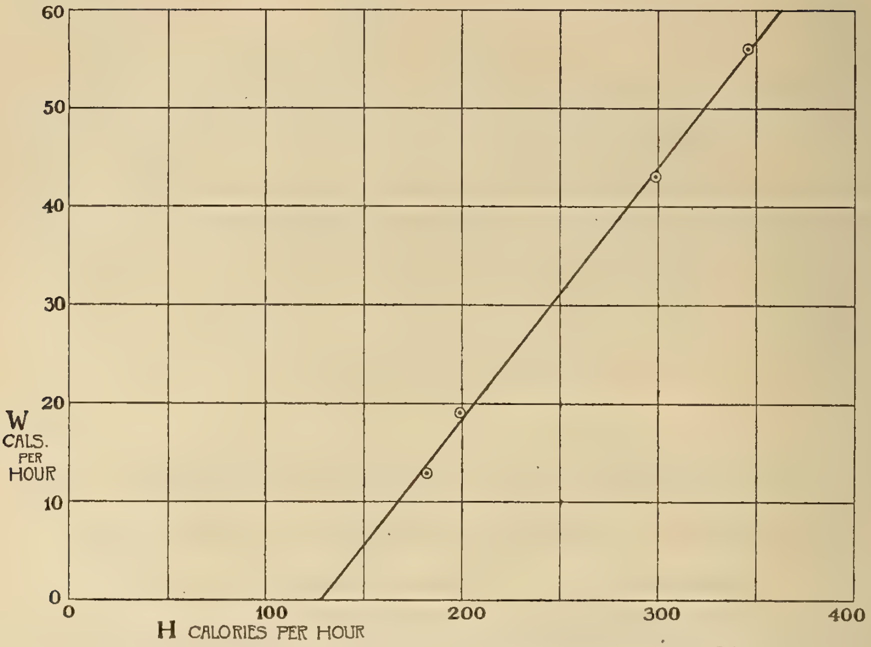

Fig. 1.—Relation between work W and mean heat produced H.

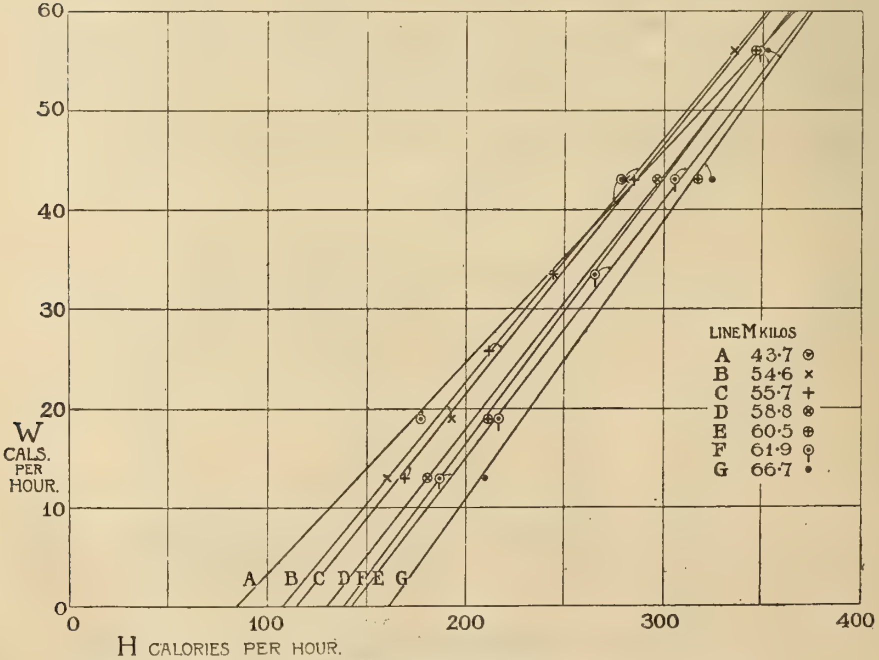

Fig. 2.—Relation between H and W for persons of various weights M.

or, more generally,
$$H = H_0 + \frac{W}{\lambda}, \qquad (2)$$

where H is the heat produced, W the work done, and H_0, λ constants which have on the average in Prof. Macdonald's experiments the values 128 and 0·256. H_0 is clearly the heat produced when the mechanical work done is zero, and arises from the motion of the limbs and the processes occurring in the body.

The fourth column of the table gives the results calculated from the formula.

But this is only an average result. It was clear from Prof. Macdonald's figures that the relation depended on the person doing the work, and we proceeded to plot the corresponding curves for the various individuals. These are shown in fig. 2; and though of course the number of observations is not

Table II.—Tabulation of Experimental Results (separated out in relation to the particular weights).

Weight, M.	Work, W.	Heat produced, H.	Measurements from curves fig. 2.	
			H_0.	λ.
kgrm.	Cals. per hour.	Cals. per hour.		
43·7	19	177	84	0·213
	43	279		
	56	346		
54·6	13	160	107	0·244
	19	193		
	43	280		
	56	335		
55·7	13	169	114	0·250
	(26)	(212)		
	(34·5)	(244)		
	43	285		
58·8	13	181	130	0·255
	43	298		
60·5	19	212	142	0·258
	43	317		
	56	347		
61·9	13	186	138	0·266
	19	216		
	(34·5)	(265)		
	43	306		
	56	348		
66·7	13	209	161	0·280
	43	324		
	56	352		

The figures in parenthesis are from a paper in 'Brit. Assoc. Rep.,' 1912, p. 286.

very large in each case for a given individual, the relation between heat and work is satisfied by a linear equation and can be expressed by the above formula, with the difference, however, that H_0 and λ depend on the individual and are not the same for all the persons tested. Table II gives the results and includes figures taken from an earlier paper in the B. A. Report for 1912.

The quantity λ measures the slope of the curve.

The next step was to investigate the relation, if any, between the quantities H_0 and λ and the weight of the man denoted by M and measured in kilogrammes. On plotting the values of λ against the mass in kilogrammes as is done in fig. 3, we found that the points again lay very well on a straight line and that the equation to this line was given by

$$\lambda = 0{\cdot}08 + 0{\cdot}003\,M. \tag{3}$$

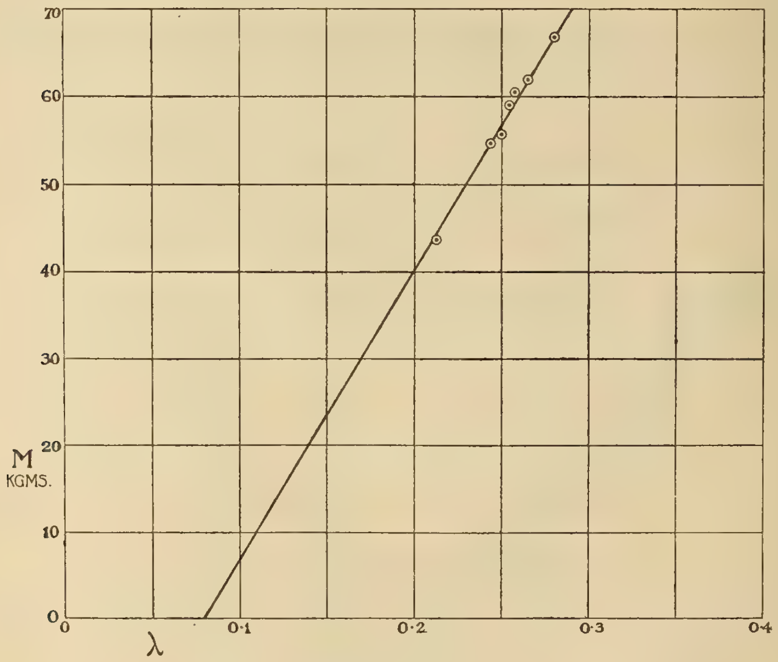

Fig. 3.—Relation between mass M and λ (slope of lines in fig. 2).

This quantity measures the ratio of the work done to $(H - H_0)$, the heat employed in doing this mechanical work, and for a man of 50 kgrm. weight has the value 0·23 or nearly one-fourth; the efficiency of such a man is about 25 per cent.

On plotting the values of H_0 against M as in fig. 4 we again found that a simple linear relation given by

$$H_0 = -138 + 4{\cdot}5\,M \tag{4}$$

satisfactorily held for all the points but one. Thus the heat a man generates by moving his limbs in a regular manner without doing external work is equal to the difference between 4·5 times his weight and a constant.

The one exception to the law was in the case of a boy weighing 43·7 kgrm. who had no experience of cycling and whose earlier experiments were omitted in consequence by Prof. Macdonald.

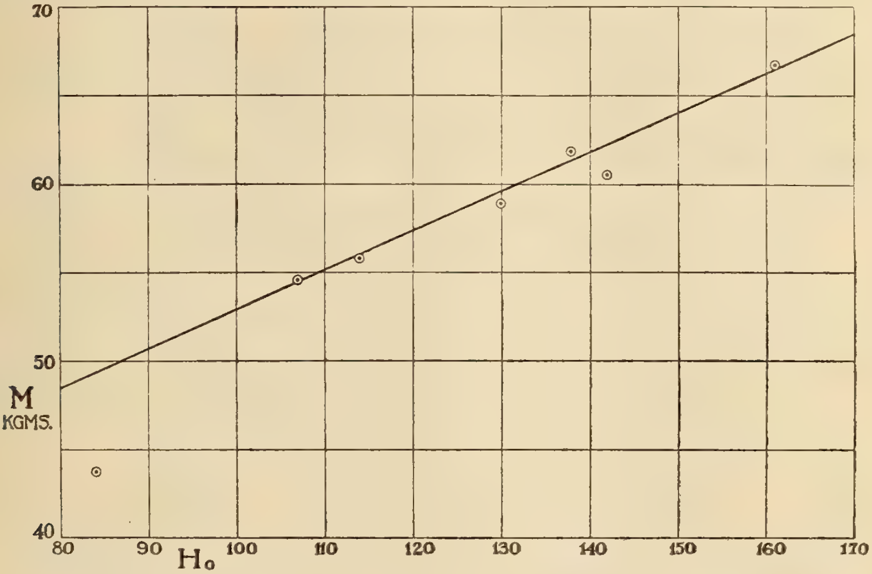

FIG. 4.—Relation between mass M and H_0 (pedals turning ; no load).

If we now sum up the results, putting the values of H_0 and λ from (3) and (4) into our formula (2), we find

$$H = -138 + 4{\cdot}5M + \frac{W}{0{\cdot}08 + 0{\cdot}003M}. \tag{5}$$

The curves obtained from this formula for different values of W are given in fig. 5, and the experimental results are there plotted.

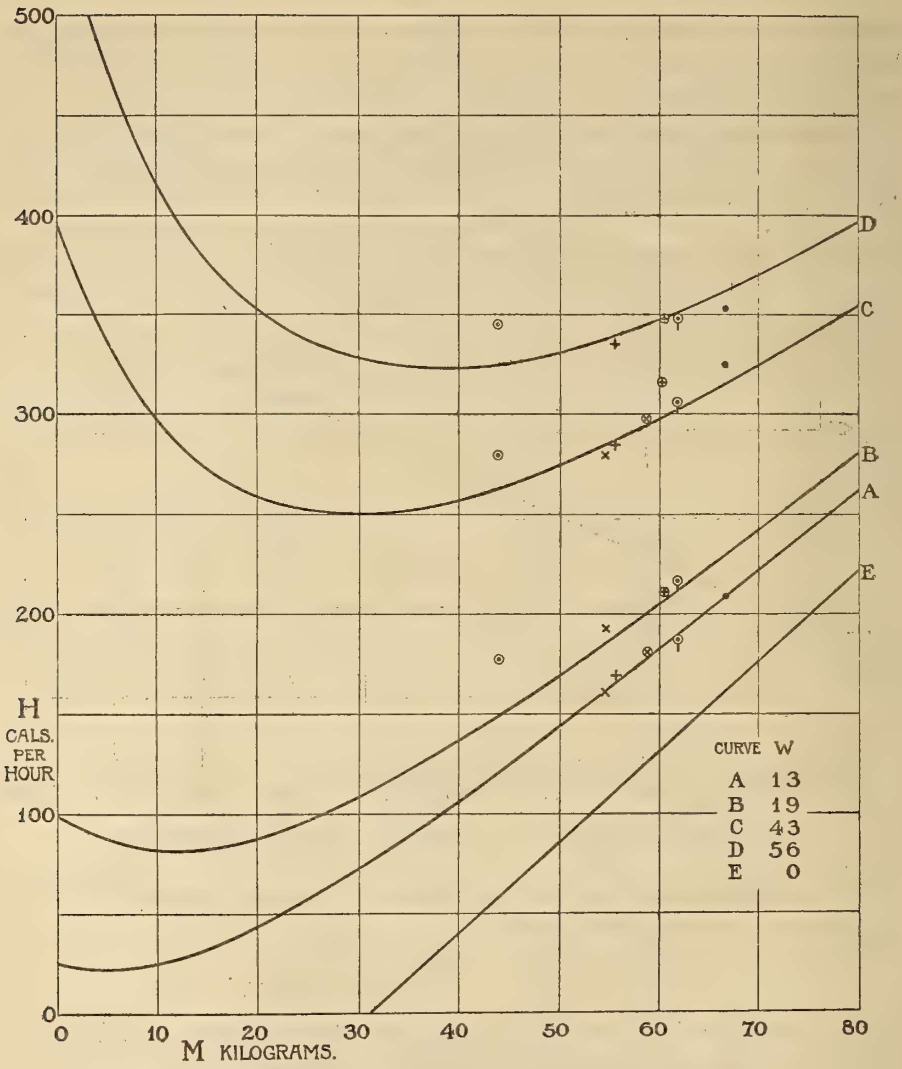

FIG. 5.—Relation between H and M calculated from equation (5).

The same results are tabulated in Table III. The differences between the results given by the formula and those found from observation are, with the exception of the boy of weight 43·7 kgrm., in no case large, and it would appear that the relation

$$H = a + bM + \frac{W}{\alpha + \beta M}, \tag{6}$$

where a, b, α, β are constants having for Prof. Macdonald's experiments the values given in formula (5), expresses, within the limits of experimental error, the relation between the work done, the heat produced, and the weight of a man. It is clear of course that the equation cannot be pressed too far; as to the value of the result found, we do not feel ourselves competent to judge. The work may, however, be of interest as an example of the analysis of somewhat complex experimental results by simple graphical methods.

Table III.—Calculated Values of H by Equation (5) for the various Constant Rates of Work, W, used in the Experiments, and the corresponding Observed Values of same.

Mass.	W = 0.		W = 13.		W = 19.		W = 43.		W = 56.	
kgrm.	Calc. H_0.	Obs. H.	Calc. H.	Obs. H.	Calc. H.	Obs. H.	Calc. H.	Obs. H.	Calc. H.	Obs. H.
0	−138		24		99		398		562	
20·0	− 48		45		88		259		352	
43·7	59		120		149	177	263	279	319	346
54·6	108		161	160	186	193	284	280	338	335
55·7	113		166	169	190		287	285	340	
58·8	127		178	181	201		295	298	346	
60·5	134		184		206	212	299	317	348	347
61·9	141		190	186	212	216	303	306	352	348
66·7	162		208	209	230		315	324	362	352
80·0	222		263		281		356		397	

On the Fossil Floras of the Wyre Forest, with Special Reference to the Geology of the Coalfield and its Relationships to the Neighbouring Coal Measure Areas.

By E. A. Newell Arber, M.A., Sc.D., F.G.S., F.L.S., Trinity College, Cambridge.

(Communicated by Prof. T. McKenny Hughes, F.R.S. Received May 20,—Read June 5, 1913.)

(Abstract.)

In the absence of any detailed knowledge of the geology of the Wyre Forest Coalfield, the area may be temporarily sub-divided into four regions. Fossil floras are described from three of these: from the horizon of the Sweet Coals in the Highley area in the north, from the unproductive beds of the Dowles Valley in the centre, and from the horizon of the Sulphur Coals of the Southern or Mamble area. On the evidence of the plants the Sweet Coal Series is shown to belong to the Middle Coal Measures, while the Sulphur Coal Series, overlying the Sweet Coals unconformably, belongs to a higher horizon, the Transition Coal Measures. The Dowles Valley unproductive measures are shown to be Middle Coal Measures, which are there over 1000 feet in thickness. The Middle Coal Measure flora of the Wyre Forest includes 55 species, of which three are new, two of them being new species of *Sigillaria*, and one, a new type of seed-impression. Four other plants are new records for Britain. The Transition Coal Measure flora is smaller, but includes 20 species, of which one is a new British record.

It is shown that both the Middle and Transition Coal Measures of the Wyre Forest Coalfield consist of red-grey measures with Espley rocks. In the Transition Coal Measures *Spirorbis*-limestones also occur. The distribution of these rocks is considered in detail.

The Wyre Forest is discussed in relation to the other coalfields of the Welsh Borderland. The lower or productive measures of Coalbrookdale, and also the coals of the Titterstone Clee Hill are shown, on the plant evidence, to be Middle Coal Measures. A species of *Cordaicladus* new to Britain is described from the latter coalfield. It is pointed out that the Coalbrookdale-Wyre Forest field really consists of four distinct coalfields, in part superimposed on one another. Two of these, the Lower Series of Coalbrookdale and the Sweet Coal Series in the Wyre Forest, are of Middle Coal Measure age. These are quite separate areas, and are in part overlain unconformably by two other coalfields of Transition Coal Measures, one connecting Coalbrookdale and the Wyre Forest, and the other confined to the southern part of the Wyre Forest, overlying Old Red Sandstone.

It is contended that the coalfields of Shrewsbury, Le Botwood, Coalbrookdale, Wyre Forest, Titterstone Clee Hill and probably Newent form a related series, which, with the exception of the Lower Series of Coalbrookdale, is quite distinct lithologically from the Midland and Southern Pennine coalfields. If this is the case, it is pointed out that the theories of the originally continuous sheet of measures, and of subsequent excessive denudation of the Welsh Borderland, are inaccurate hypotheses, which should be abandoned.

Intermittent Vision.

By A. Mallock, F.R.S.

(Received November 11,—Read December 11, 1913.)

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The Determination of the Minimal Lethal Dose of various Toxic Substances and its Relationship to the Body Weight in Warm-Blooded Animals, together with Considerations bearing on the Dosage of Drugs.

By Georges Dreyer, M.D., Fellow of Lincoln College, Professor of Pathology in the University of Oxford; and E. W. Ainley Walker, D.M., Fellow and Tutor of University College, Lecturer in Pathology in the University of Oxford.

(Communicated by Prof. C. S. Sherrington, F.R.S. Received November 22, 1913,
—Read January 22, 1914.)

(From the Department of Pathology, University of Oxford.)

In the course of investigations on the production, distribution, and rate of disappearance in the body of immune substances, we were occupied in 1908 and previous years with a series of experiments on agglutinins, and we arrived at conclusions pointing to their close relationship to the blood and blood-forming organs (1, 2). In association with these inquiries, one of us (G. D.), together with W. Ray, published a communication on the relationship between the blood volume and the distribution of agglutinins within the circulation (3).

It was there shown that the concentration of this substance (agglutinin) in the blood after inoculation into an animal was proportional to the body surface of the animal concerned, and was thus approximately proportional to the two-thirds power of the weight. Hence was deduced the conclusion that the blood volume of the animals examined was proportional to their body surface.

The recognition of this relationship between surface and blood volume made it clear that the assumptions hitherto made use of in attempting to determine dosage for the administration of therapeutic substances such as antitoxins and drugs required complete revision, at any rate, in so far as the activity of these substances might be supposed to be dependent on their concentration in the circulating blood. Subsequently the surface relation ($W^{\frac{2}{3}}$) was taken up by B. Moore (4) in an interesting communication dealing with the dosage of drugs, and especially with the therapeutic action of atoxyl and various other compounds of the heavy metals in the treatment of trypanosomiasis. Moore came to the conclusion that, in regard to these drugs, tolerance was limited by the surface area of the mucous membrane of the alimentary canal. This, in his opinion, offered a valid explanation of the fact that it is often difficult or impossible to administer effective doses of these drugs to large animals, since these animals do not tolerate the doses which would be required to produce the same concentration in their blood as is needed for successful therapeutic action in small animals.

The observations on agglutinins had already led us to the conclusion that the concentration of inoculated drugs and other foreign substances distributed in the blood plasma would necessarily be proportional to the surface of the animal, since it was shown that the blood volume was always proportional to the body surface. And this fact would equally apply within any given species to an internal surface such as that of the alimentary canal.

The blood volume formula had, however, immediately and seriously been called in question (5, 6). Accordingly, it was necessary to turn aside from the problems in hand until the criticisms offered had been carefully examined, and the relationship of blood volume to the surface area had been adequately confirmed. This, so far as we are able to judge, has now been done (7).

Accordingly, we now proceed on the assumption that the blood volume of warm-blooded animals is a function of their body surface, and is given by the formula $B = W^n/k$, where n is approximately 0·72, and k is a constant to be ascertained for each particular species of animal.

Using this assumption, we find that the minimal lethal dose of a long series of substances of widely different origin and composition can satisfactorily be expressed as a function of the body surface. The series of substances which have been found to follow this law includes not only organic bodies both of animal and vegetable origin, but also a number of inorganic compounds.

It follows that we are entirely in accord with the main conclusion reached by Moore in his very suggestive discussion of the subject, namely, that dosage must be proportional to body surface (in warm-blooded animals). But, in view of the results which follow from the application of the blood volume formula, we are unfortunately not in a position to agree with the line of argument by which he deduces this conclusion from a consideration of the area of the alimentary tract. Moreover, we are quite unable to admit as satisfactory the explanation which he offers of the fact that drugs which are successful in the treatment of various species of small animals are not successful in the case of species of large size in any dose which can be used with safety. For reasons which will be given below, the explanation of these facts appears to us to be dependent on a specific tolerance or intolerance, as the case may be, in different species of animal, and not upon the difference in their size or relative area of alimentary surface as suggested by Moore.

In proceeding to discuss our own examination of the subject of the present communication, we begin by endeavouring to show how the dose of a given poison which kills animals of a particular species in a given time is related to the weight of the individual.

For this purpose certain experiments with diphtheria toxin, the full details of which will be found in a subsequent paper ("An Analysis of the Problem of the Minimal Lethal Dose, etc."), are made use of, and some of the results obtained are given below.

In the case of diphtheria toxin, if the death time (3½ days) which is usually taken in the standardisation of this substance be made use of, it is found that for guinea-pigs which die in about 80 hours the lethal dose expressed as a percentage of the weight works out as follows :—

For animals of between—
200 and 250 grm. weight, about 6.0 cu. mm. of Toxin B per 100 grm.
310 „ 370 „ „ 5·2 „ „ „
415 „ 530 „ $<$ 5·0 „ „ „

Again, taking a death time of some 40 hours, it is found that in the lightest group of guinea-pigs the minimal lethal dose per 100 grm. of weight is about 6·5 cu. mm. of the toxin, in the group of medium weights it is about 6·2 cu. mm., while in the heaviest group it is about 6 cu. mm.

Hence it follows that, for individuals of differing weights, the minimal lethal dose cannot be rightly expressed as a percentage of the body weight. This fact is, of course, well known to those familiar with the routine estimation of toxicity.

In Tables I and II are given two groups of animals, the one group consisting of light individuals and the other of heavy ones; where the dosage expressed in per cent. of body weight was approximately the same.

The average weight of the animals in Table I is 234 grm., the average dose per 100 grm. is 6·3 cu. mm., the dose estimated in relation to the surface area, and calculated from the expression $D = d/W^{0\cdot72}$, where d is the actual quantity of the drug introduced, is 29·1 cu. mm., and the average time to death is 46 hours.

The average weight of the animals in Table II is 425 grm., the average dose per 100 grm. is again 6·3 cu. mm., but the dose calculated in relation to the surface area has increased to 34·3 cu. mm. and the average time to death is seen to be reduced to 37 hours. It is, therefore, evident that when the dose per 100 grm. of weight is made the same in light and heavy groups of

Table I.—Experiments with Diphtheria Toxin B in Guinea-pig (subcutaneous injection).

Group of Light Animals.

No.	No. of experiment.	Weight of animal, in grm.	Actual dose d, in c.c.	Dose (D) in relation to surface × 10^7. $\text{D} = d/\text{W}^{0\cdot72}$.	No. of hours to death.	Dose in per cent. of weight, cu. mm. per 100 grm.
1	44	215	0·01400	2940	42	6·51
2	43	230	0·01375	2745	64	5·99
3	42	255	0·01575	2920	40	6·19
4	45	235	0·01535	3020	38	6·53
Average.........		234	0·01471	2906	46	6·31

Table II.—Experiments with Diphtheria Toxin B in Guinea-pig (subcutaneous injection).

Group of Heavy Animals.

No.	No. of experiment.	Weight of animal, in grm.	Actual dose d, in c.c.	Dose (D) in relation to surface × 10^7 $\text{D} = d/\text{W}^{0\cdot72}$.	No. of hours to death.	Dose in per cent. of weight, cu. mm. per 100 grm.
1	28	425	0·02780	3560	30	6·55
2	32	415	0·02715	3525	49	6·54
3	31	435	0·02845	3580	30	6·54
4	35	415	0·02480	3220	44	5·98
5	30	435	0·02590	3258	32	5·96
Average.........		425	0·02682	3429	37	6·31

animals of the same species the lighter animals survive for a much longer period than do the heavier. The explanation of this difference in death time is to be sought in a comparison of the doses calculated in relation to the surface. It is then seen that the dose thus calculated is much smaller in the lighter animals than in the heavier group.

That this is a valid method of calculating dosage follows from the fact that, under ordinary conditions, substances administered as drugs, to act after absorption into the body, must become diluted in proportion to the volume of the blood. They are carried to the tissues of the body through the medium of the plasma in a relative concentration which is determined by the volume of the circulating blood. But the volume of the blood is a function of the body surface. Accordingly, it follows that the concentration in the plasma of any substance administered to animals under like conditions in doses proportional to their body weights will be much less in the lighter animals than in the heavier individuals of the same species.

On the other hand, if the doses be administered in relation to the body surface, their initial and their maximal concentration in the plasma will be the same whatever be the weights of the individual animals concerned.

The results brought forward for diphtheria toxin do not constitute an isolated instance in support of this view, that in any given species of animal dosage must be used in relation to the volume of the blood. Very numerous observations from the literature of toxicology which we have collected and analysed confirm the accuracy of this method of measurement. It appears to hold, so far as we have been able hitherto to ascertain, for a large number of substances of very different constitution and of diverse mode of action in warm-blooded animals. Wherever a sufficient number of accurate data can be found the effect of dosage can be shown to be related to blood volume and surface area in any given species. Numerous results which have been thought to be inexplicable when the dosage was expressed in per cent. of body weight, except on the ground of special individual susceptibility or individual resistance, in reality give precisely the results which would have been expected had the dosage been expressed in terms of body surface.

In the case of arsenic (As_2O_3) in the rabbit the observations of Morishima (8) afford an interesting illustration. The data and the calculations from these observations are given in Table III. Here it is seen that the time of death shows no exact relation to the dose expressed in per cent. of weight, but it follows quite closely the dose in relation to surface, though animal 5 shows an irregularity in living longer than animal 4. It will, however, be seen that the average dose per surface of animals 3 and 5, taken together, and their average time of death are identical with

Table III.—Arsenic (As_2O_3) in Rabbit, Morishima's Experiments (subcutaneous injection).

No.	Weight of animal, in grm.	Actual dose (d), in mgrm.	Dose (D) in relation to surface, in mgrm. $D = d/W^{0 \cdot 72}$.	No. of hours to death.	Dose in per cent. of weight, mgrm. per 100 grm.
1	1324	8·61	4·86	∞	0·65
2	1103	7·72	4·95	∞	0·70
3	1495	10·47	5·42	84	0·70
4	1112	8·90	5·67	96	0·80
5	1702	11·90	5·91	108	0·70

the surface dose and time of death of animal 4, while the doses in per cent. of weight differ by 14 per cent. Again, if we compare the three animals which received equal doses, if the dose is expressed in per cent. of weight (viz., 2, 3, 5), the lightest one survives while the two heavier ones succumb. The explanation is at once evident on comparing the size of the doses expressed in relation to surface.

In Table IV is given another series of observations by Morishima where the injection was made intravenously. This method is, of course, likely to yield more precise results than subcutaneous inoculation, and it is seen that the effects of the doses, when the latter are expressed in relation to body surface, are remarkably regular and striking. On the other hand, when the dose is given in percentage of body weight, as was done by Morishima himself, the time to death varies very widely in animals which received equal dosage with the drug on his method of calculation. Morishima's results might be taken to indicate great individual differences in susceptibility in different individual animals. But such individual differences do not appear when the dose is calculated in relation to blood volume and body surface.

Table IV.—Arsenic (As_2O_3) in Rabbit, Morishima's Experiments (intravenous injection).

No.	Weight of animal, in grm.	Actual dose (*d*), in mgrm.	Dose (D) in relation to surface, in mgrm. $D = d/W^{0.72}$.	No. of hours to death.	Dose in per cent. of weight, mgrm. per 100 grm.
1	1135	6·81	4·30	∞	0·60
2	1190	7·73	4·71	432	0·65
3	970	6·79	4·81	48	0·70
4	1155	8·08	5·04	21	0·70
5	1952	13·66	5·82	8	0·70

Similar facts can readily be made out from various other experiments which have been carried out with arsenical compounds by a number of observers.

In the case of another heavy metal, cobalt, the same results hold good when the dosage is expressed in relation to surface instead of in the usual manner as a percentage of the body weight. This fact has been determined by an analysis of Meurice's experiments (9) on pigeons injected into the breast muscles with cobalt nitrate, $Co(NO_3)_2$. This is of special interest in view of the fact that it has already been shown in the experiments which we carried out in association with H. K. Fry, referred to elsewhere (10), that the blood volume of birds (like that of mammals) is proportional to their surface area.

Without further multiplying detailed instances it may be stated that we have obtained the same results on calculation from a variety of published observations on a number of different substances administered by various methods in different animals. Among these substances are *sulphate of methyl brucium* injected subcutaneously in the rabbit, and *codeine hydrochloride* administered by the stomach in the same animal (Crum Brown and Fraser (11)); *sulphate of physostigma* given subcutaneously in rabbits (Fraser (12)); *morphia* and *atropine sulphate* administered subcutaneously in the rat (Bashford (13)); various *snake venoms*—krait, *Enhydrina valakadien*, *Enhydris curtus*, cobra—inoculated in rats, rabbits, guinea-pigs, and cats by different observers (Fraser and Elliott (14), Elliott, Siller, and Carmichael (15), Madsen and Noguchi (16), and others); *adrenalin* both natural and synthetic in the mouse (Schultz (17)); *tetanus toxin* injected subcutaneously in the mouse (Knorr (18)); *potassium chloride* (KCl) introduced intravenously in the rabbit (Hald (19)); and *caffeine* subcutaneously, intraperitoneally, intravenously, or by the mouth in dogs, cats, rabbits, and guinea-pigs (Salant and Rieger (20)).

In view of the conclusions to which the results obtained with all these very diverse toxic agents lead, it seems clear that in animals of different size in any given species the dose required to produce a given effect is related to the surface and blood volume of the animal and not directly to the body weight. That is to say, the smaller individuals require a relatively larger dose than the heavier animals to produce the same effect.

While we are not prepared to maintain that this constitutes a *universal* rule to which there are no exceptions, yet it follows from what has been already stated that it possesses a very wide application, and we have not up to the present met with any exception in the case of mammals and birds.

Accordingly we conclude that if it is desired to administer comparable doses of drugs in warm-blooded animals of different size and weight in any given species, they must be calculated in relation to the body surface.

It follows from this that if one administers any given toxic substance in doses such as will kill each of the animals employed in about the same period of time, one is now in a position to use animals of various size over a wide range of weight within the same species instead of only animals of one particular size in carrying out experimental work upon toxicity and lethal dosage. One is no longer restricted to the use of carefully selected animals of a given and standard weight, as has hitherto been the case, for example, in all determinations of the strength of toxins as well as in the standardisation of antitoxins. This result will naturally prove of value in facilitating toxicological investigation in very many directions.

In case of *cold-blooded animals* we are not at present able to put forward any definite statement; but the problems which they present are under investigation.

As regards the influence of *sex* in warm-blooded animals, we find an indication in our figures that female animals require a somewhat smaller dose to produce a given effect than male individuals of corresponding weight. This agrees with what has frequently been pointed out as the result of clinical experience. The observation seems to find its explanation in the fact that the blood volume of female animals is slightly smaller (7) than that of males. For both the initial and the maximal concentration in the plasma of any drug administered by a given route in a series of animals of different size and weight will naturally be related to the volume of the plasma. Whatever be the rate at which it is selected from the plasma by particular cells, and whatever be the rate of its elimination from the body, the concentration in the blood plasma of any given substance must at every stage be related to the volume of that plasma in the individual animal concerned. Thus a given dose of any substance administered (in one and the same dilution) will reach a higher effective concentration in those individuals whose blood volume is less than in those in which it is greater.

The importance of this question of concentration may be illustrated by a reference to the experiments carried out by Hald (19) with potassium chloride. These showed that in individuals of equal weight the effect of one and the same dose of the active substance was greater, and manifested itself more rapidly, the higher the *concentration* in which it was given.

In view of these considerations it becomes of interest to return to the question of the failure encountered in the treatment of trypanosomiasis in large animals with drugs successfully employed in the smaller species.

If one compares the doses necessary to produce the same concentration of a given drug in the plasma of man and the rat, it can readily be shown that even if a man of 70 kgrm. could be given the same dose per kilogramme as a rat of 140 grm. weight—the figures selected by Moore in his discussion—the concentration of the drug in the man's blood plasma would only be about 75 per cent. of that obtained in the rat. Accordingly, the same therapeutic effect could not be produced. Man, however, cannot tolerate anything approaching this degree of dosage, and hence the treatment which is curative in rats becomes inapplicable in the human subject. But even these facts do not, as it seems to us, afford the whole explanation of the difficulty in question. For it appears that differences in tolerance and intolerance to particular substances in different species of animal are of a specific character and cannot be explained merely by relative differences in blood volume and body surface.

In proof of this, attention must be drawn to the fact that it is not always the larger species which are more susceptible than the smaller species to dosage proportional to their relative body surface, or even to their relative body weight. Sometimes the conditions are reversed. Thus, as is well known, a horse infected with tetanus may be found in apparently excellent condition and as yet exhibiting no symptoms of the disease at a period when its blood already contains enough tetanus toxin to kill a guinea pig injected with only a few cubic centimetres of the horse's serum. Similarly in the case of rats and guinea pigs, rodents of about the same size, the rat can resist several hundred times the dose of diphtheria toxin which will be fatal to the guinea pig within a few days.

In the case of substances other than bacterial toxins similar examples showing a greater resistance in the larger species than in the smaller can readily be found, as for instance in the experiments of Meurice, already referred to (9), in Bock's experiments (21) with cobalt compounds, in Jodlbauer's paper on Tetramethyl ammonium chloride (22), in Fraser and Elliott's experiments on Cobra venom and on Enhydrina venom (14), and in many other pharmacological investigations. It follows that drug susceptibility is by no means necessarily greater in the larger species than in the smaller, but on the contrary it is frequently less. Accordingly, any general explanation of drug action in different species of animals, which is based upon the relative size of their surface, cannot be maintained. Only within one and the same species of animal will the surface relation prove a reliable guide in dosage.

In this connection it is of some interest to consider briefly formulæ for dosage in the human subject such as have been made use of or suggested by various writers. For the sake of ease in calculation these have usually been based on the age of the patient, and most of them appear to aim at giving dosage in relation to the body weight.

But in the case of the formula of Thomas Young, 1813, we meet the earliest example of dosage calculated so as to give younger individuals a relatively greater dose per unit of body weight than is given to adults. Young wrote that "for children under twelve years old, the doses of most medicines must be diminished in the proportion of the age to the age increased by twelve: for example at two years old $1/7 = 2/(2 + 12)$. At twenty-one the full dose may be given. Y." (23).

We owe the exact reference to the kindness of Dr. A. J. Jex Blake; but how Young arrived at his formula, $\text{Age}/(\text{Age} + 12)$, it has not been possible to discover from his writings. However this may be, his formula actually gives for all ages from about four or five to about 16 a dosage fairly approximating to dosage by the surface area. But below the age of five years the dosage by Young's formula falls more and more rapidly below the dose calculated in relation to body surface.

We append a table showing the doses which would be given at different ages from 1 year to 21 years in a system of dosage calculated in relation to blood volume and body surface, taking the weights at the different ages as given in Vierodt's tables, 1893.

Table of Dosage.

Age, in years.	Average weight, in grm.	Dose in relation to surface.	Dose as a fraction of dose for adult.
21	61,200	100·0	$\frac{1}{1}$
20	59,500	98·8	
19	57,600	95·7	
18	53,900	92·5	
17	49,700	86·2	
16	45,400	81·4	
15	41,200	75·1	$\frac{3}{4}$
14	37,100	70·1	
13	33,100	64·7	
12	29,000	58·3	
11	27,000	55·4	
10	25,200	52·8	
9	23,500	50·6	$\frac{1}{2}$
8	21,600	47·2	
7	19,700	44·6	
6	17,800	41·1	
5	15,900	38·1	
4	14,000	34·3	$\frac{1}{3}$
3	12,500	31·7	
2	11,000	29·2	
1	9,000	25·1	$\frac{1}{4}$
0	3,100	11·8	$\frac{1}{10}$

In the above table the dose in relation to the surface is given as calculated from the body weight, and points are indicated where the calculated dose approximates to a simple fraction of the adult dose. These work out extremely conveniently for practical application. Thus at 15 years (approximately three-quarters of the adult age of 21) the dose is $\frac{3}{4}$; at 9–10 years (nearly half the adult age) the dose is $\frac{1}{2}$; at 3–4 years it may be given as $\frac{1}{3}$; at 1 year of age it is $\frac{1}{4}$; below that age it sinks to as little as $\frac{1}{10}$ of the adult dose.

In conclusion we would draw attention to the fact that as long ago as 1818 Hufeland (24) gave the dose at fifteen years as $\frac{3}{4}$ and the dose at one year as $\frac{1}{4}$, though he placed the half dose at six years of age instead of at nine or ten as our table makes it. Thus it appears that both he and Young already recognised the necessity of giving relatively larger doses than would be proportional to body weight in the younger and smaller individuals of the species. On the other hand Dilling (25) in a recent communication proposes a formula which approximates roughly to a dosage per kilogramme of body weight. This is a system of dosage the fallacy of which was emphasised by Moore. In view more especially of the considerations brought forward above, we venture to suggest that it should now be entirely abandoned.

Conclusions.

1. In warm-blooded animals of the same species but of different weights dosage must be calculated in relation to the body surface.

This result agrees with the conclusion already reached by Moore* though on different grounds.

2. This statement is to be explained on the ground that the concentration in the plasma of any given substance administered is dependent on the volume of the circulating blood, which is itself proportional to the body surface in any given species of animal.

3. It follows that in the accurate measurement and standardisation of toxic substances and antitoxins it will now be possible to make use of animals of different weights within a given species instead of using only animals of an arbitrarily selected weight, as has hitherto been necessary.

4. Results in dosage calculated from one species of animal cannot directly be applied to another species merely by taking surface into due consideration, since tolerance and intolerance are specific characters which are shown to be in many cases independent of the size of the species concerned.

5. For the human subject dosage in relation to the surface works out very simply as approximately :—

At 21 years.........	Full dose	At 3–4 years	$\frac{1}{3}$ dose
,, 15 ,, ...	$\frac{3}{4}$,,	,, 1 ,, ...	$\frac{1}{4}$,,
,, 9–10 ,, ...	$\frac{1}{2}$,,	In the early months ...	$\frac{1}{10}$,,

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* Moore's conclusions seem to rest in the main on a consideration of "substances which act by stimulation or inflammation of surfaces."

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*Experiments on the Restoration of Paralysed Muscles by Means of Nerve Anastomosis.** Part II.—*Anastomosis of the Nerves supplying Limb Muscles.*

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(Communicated by Prof. J. G. McKendrick, F.R.S. Received November 25, 1913,—Read January 22, 1914.)

(Abstract.)

The part of the research into the anastomosis of nerves dealt with in this paper has reference to the restoration of function of a group of muscles in the limb. Following the early experiment of Flourens, several workers (Rawa, Stefani, Howell and Huber, Cunningham, and the author) published investigations on the effects of nerve crossing, or the division of two nerves in the limb and cross suture of the ends.

These investigations, while showing that restoration of function can take place through the composite nerve, and that even in the cerebral cortex the areas associated with flexion and extension become interchanged, left it doubtful whether in the event of one nerve being eliminated, the muscles supplied by it could be innervated by a neighbouring motor nerve, which at the same time could continue also to supply the muscles proper to it, performing thus a double function.

The subject was investigated from this point of view by Kilvington, who published a series of experiments in which the external popliteal nerve was cut and the peripheral segment anastomosed to the internal popliteal, and *vice versâ*. He reports recovery of function after this procedure.

Doubt, however, still remains as to the possibility of an extensor and flexor group of muscles recovering the capacity of performing co-ordinated movements under such conditions, as in the case of the hind limb of the dog even when the sciatic nerve cut high in the thigh has not united, the animal is able to use the leg in walking, the chief defect being that it walks on the dorsum of the paw. Also, in these circumstances, the foot is sometimes, possibly by accident, placed plantar surface down. Therefore the reported recoveries after such experiments leave doubt whether the recovery is real or apparent. In addition, it is impossible to investigate the changes in the

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* The expense of this research has been defrayed by a Government Grant from the Royal Society.

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cortical representation in the case of the hind limb, as in the dog this is represented by a single centre.

The author has performed five experiments in dogs on the right fore-limb, which has the advantage, in the first place, that section of the nerves above the elbow paralyses the limb in such a way that it is impossible for the animal to use it for walking until recovery of co-ordinated movements occurs. This is then a very severe test. In the second place, the fore-limb of the dog is represented by two separate and distinct centres, one for flexion and one for extension, which are only exceptionally defective.

The experiments are of two kinds, but are the same in so far that in all the musculo-cutaneous, median, ulnar, and musculo-spiral nerves were each divided above the elbow, and the limb thus completely paralysed below the elbow. Then the four distal segments were united to the proximal end of the musculo-spiral nerve, and the three remaining proximal ends left ununited; or, on the other hand, the four distal segments were united to the proximal ends of the musculo-cutaneous, median, and ulnar, and the one remaining proximal end (musculo-spiral) left ununited. The limb was fixed in plaster of Paris as long as necessary.

The following results were obtained:—

A. Where the central supply was that proper to the flexor muscles (musculo-cutaneous, median, and ulnar), the first sign of recovery of the muscles was shown at 96 and 93 days respectively after the operation, and a satisfactory recovery, enabling the dog to run about, and in one case to be taken for exercise into the streets, was reached at 126 and 123 days respectively. In the first case no interruption of the excellent recovery occurred as long as the animal was allowed to live, namely, 225 days, but in the latter case there was some trouble by the development of a slight flexor contracture which hindered the recovery up till the animal was killed at 187 days.

B. Where the central supply was that proper to the extensor muscles (musculo-spiral), the first sign of recovery was at 81 and 59 days respectively in the two which showed recovery. In the first case a pressure sore interfered with further progress, but in the second case at 79 days the animal ran about normally, and was able to be taken out for exercise in the streets without any fear of attracting notice. It may be noted that although in this form of experiment the muscles of the limb were supplied by the lesser number of nerve fibres, namely, those contained in the musculo-spiral, the recovery commenced earlier than when the three nerves normally supplying flexors formed the sole central supply, although in that case the greater number of nerve fibres were available for the supply. This is explained as due probably to the earlier recovery of the extensors when the musculo-spiral was the common source of supply, thus enabling the leg to be earlier held extended, and therefore useful for walking.

Each experiment was investigated before the animal was killed, as follows:—

A. *Examination of the Nerves.*—In every case the union of the nerves had taken place as intended, *i.e.* no reunion of any nerve intended to be eliminated had occurred. Also it was found that when the musculo-spiral trunk was supplying both flexors and extensors, it conveyed the fibres for the flexors and those for the extensors along different sides of the nerve, where they could be separately stimulated.

B. *Examination of the Brain.*—In every case in which this examination was able to be made, namely, in one of the first type of experiment and in two of the second, the centre at which stimulation normally produces contraction in the muscles of the eliminated nerve supply was inexcitable, and in the other centre (either the normal flexion or the normal extension centre according to the type of the experiment) both flexion and extension were evoked, and at no point in the centre could separation of these movements be obtained.

The operation which Nicoladoni introduced for cases in which infantile paralysis has destroyed the function of a group of muscles presents the same problem as do cases of nerve anastomosis. This operation consisted of substituting for the lost muscles a portion of a neighbouring muscle so as to regain some of the lost function, and if the lost function or a part of it can be thus regained, it can only be by the nerve supply of the muscle from which the substitute is taken altering its function so as to cause the movement proper to the paralysed muscle instead of that normally belonging to it.

A case in which the author performed this operation was carefully examined in order to exclude sources of fallacy. The function of the extensors in the leg was lost and a talipes equinus by contraction of the gastrocnemius was present. This was in a girl aged 7, and had lasted for six years. The extensor muscles gave no reactions to electrical tests. At the operation the gastrocnemius was lengthened to overcome the talipes, and one-third was taken from the outer part of that muscle and attached in front to the tendons of the paralysed muscles. Sixty-nine days after the operation the patient had power to extend the foot voluntarily, some eversion being produced at the same time owing to the line of action of the new muscle. The new muscle also stood out as a tense band while the voluntary movement was being performed. The two separate movements were also able to be evoked by galvanic or faradic stimulation over the two separate parts of the gastrocnemius. After the recovery of voluntary extension a further examination of the extensor muscles was made, and this showed that the movement of extension was not made by the extensor muscles, as they were not able to be stimulated. A platinum electrode was also inserted into the extensor muscles through the skin, but no contractions could be produced in them either by the galvanic or by the faradic current.

The following are the conclusions from the research :—

(1) In the limb of the dog when the nerve supply of one group of muscles is eliminated, the nerve supply of its antagonistic group may be used to supply both groups, and under these conditions co-ordinated movements may be restored.

(2) When two antagonistic groups of muscles in the limb of the dog have their nerve supplies cut and both groups then made to derive their supply from that of the one group, the group whose nerve supply is utilised probably will be the first to recover.

(3) Recovery of function of antagonistic muscles is slower to occur when one nerve supply is eliminated than in the case of nerve-crossing experiments where no nerve is eliminated, but where the supply of the two groups is crossed: and this delay is caused by reduction in the former case of the total volume of the nerves supplying the limb, and possibly by greater difficulties of adaptation in the brain to the new conditions.

(4) Where in the dog one nerve has been made to supply not only its own but also the antagonist of its own muscle, the nerve fibres passing to the two muscles in the nerve trunk proximal to the junction may be so completely separated that it may be possible to stimulate each group without affecting the other, producing thus at will contraction either of the one or of the other muscle, both being now supplied by a single central trunk.

(5) When two groups of antagonistic muscles in the limb of the dog are represented by separate cortical areas, and when the nerve supply of one of the groups is eliminated, both groups being caused to be innervated by the remaining nerve supply, the cortical area corresponding to the eliminated nerve supply becomes inexcitable, while the other cortical area on stimulation causes contraction in both groups of muscles.

(6) Where one group of muscles is paralysed, and a portion of an antagonist muscle is detached from its insertion and attached to the tendons of the paralysed group, this substitute for the paralysed group may enable the function of that group to be performed to a certain extent, and the function recovered by means of this procedure is probably controlled by the same adaptation in the central nervous system as occurs in the case of nerve anastomosis.

(7) The adaptation in the central nervous system which allows restoration

of function to take place after nerve anastomosis is not due to a simple re-education process, as there is no evidence of this during recovery, but is probably due to an alteration in the centres under the influence of altered afferent impulses from the muscles, the brain thus having the capacity quickly to adapt itself to such alteration.

Variations in the Sex Ratio of Mus rattus *Associated with an Unusual Mortality of Adult Females.*

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(Communicated by Prof. C. J. Martin, F.R.S. Received November 28, 1913,—Read January 22, 1914.)

At the commencement of June, 1911, whilst engaged on plague epidemiological observations in the United Provinces my attention was drawn to the fact that nearly all the young *Mus rattus* that were being trapped and examined by our staff in Lucknow were females. It was this strange phenomenon, the parallel of which I had never encountered during a five years' experience of plague research in India, that prompted the inquiry, the results of which are briefly set forth in this paper.

A few words explanatory of the methods employed in the daily routine examination of rats will show the nature of the material at my disposal. The prime object in trapping and examining large numbers of rats was, of course, to ascertain how far facts thus collected would assist us in solving the plague epidemiological problems with which we were faced. The species, sex, and weight in grammes of each rat caught were noted; the address of the house in which the rat was trapped and the number and species of fleas found on it were recorded. The sex of each rat was noted after dissection of the animal, and if it were female a further note was made as to the existence of pregnancy. If pregnant the number of fœtuses was likewise written down. Finally, any pathological or other condition calling for comment was fully described.

All this information, which was in the first place recorded on cards, one card for each rat, was at the end of the day's work entered in a register. Weighing the rat was done in a specially constructed spring balance, by means of which the weight in grammes to the nearest multiple of 10 could be readily and accurately ascertained. I wish to emphasise the fact that the sex of the animal was noted only after dissection, so that mistakes under this head were very unlikely to occur. A young female is not always easily differentiated from a young male by external inspection only.

Under ordinary circumstances *M. rattus* would appear to be slightly polygamous. Some observers have stated that it is very markedly so, and explain the fact that about equal numbers of males and females are usually caught by the alleged shyness of the adult female. This point I have carefully gone into and believe the allegation has no foundation in fact. As will be shown later, about equal numbers of males and females appear to be born under normal conditions. When adult age is reached there is either a somewhat enhanced mortality amongst males as compared with females or else males are more wary and so less readily caught. It should be noted also that females are as readily caught at the height of the breeding season as at other times.

In the presence of severe plague a condition of marked polygamy is sometimes met with. I believe this to be chiefly due to the fact that plague is a more fatal disease to male than to female rats.* When polygamy is marked rats are scarce or difficult to trap; on the other hand, when the rat population is very large the numbers of the two sexes trapped appear to be more nearly equal. In support of these statements Tables II–V have been produced.

(1) Ballia district: Here plague is always present and rats, probably in consequence, are very difficult to catch. Out of 4525 *M. rattus* caught during 10 months 2550 were females, a proportion of 77 males for every 100 females. If the rats be separated into two groups, young and adult, considering half the rats of 90 grm. and all those of lesser weight as young and the remainder as adults, the degree of polygamy prevailing amongst the rats of Ballia is seen to be even more marked than the above figures indicate. Of the 1875 young rats 943 were male and 932 female, whereas of the 2650 adult rats 1618 were female and only 1032 male. In other words, there were only 63 adult males for every 100 adult females (see Table V). The rats were obtained from places scattered all over the district.

(2) Coimbatore Town: Has suffered from repeated but not very severe epidemics of plague. *M. rattus* appears to be scarce; the catches were very small. Here of 3889 *M. rattus* 2072 were females, 88 females for every 100 males (see Table IV). Here again the excess of females over males affects adults only.

(3) Cawnpore: Here rats were extraordinarily numerous. When our observations started a mild epidemic of plague was drawing to a close. No acute rat plague was, however, met with. During the previous few years

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* See 3rd Plague Report, 1907, 'Journ. Hygiene,' vol. 7, p. 750, and 7th Plague Report, 1912, 'Journ. Hygiene,' vol. 12, p. 265, Table VII.

the city had almost completely escaped plague, though in the more remote past its epidemics had been extremely severe. Our very high catches indicated a very large rat population and a complete recovery from the onslaughts of plague. In Cawnpore of 51,181 *M. rattus* examined in one year 25,838 were females, *i.e.*, 98 males for every 100 females (see Table II).

(4) Banda Town has never suffered from epidemic plague. *M. rattus* was present in very large numbers. In Banda of 10,127 rats caught during 11 months 5174 were females, *i.e.*, 96 males for every 100 females (see Table III).

In Lucknow, in which city the very abnormal conditions, fully described below, were present, of 34,908 *M. rattus* caught during the course of one year 18,396 were females, *i.e.*, 89 males for every 100 females (see Table I).

Disturbance in the Sex Ratio of M. rattus *in Lucknow and its Readjustment.*

A reference to Table I will indicate the nature of the phenomenon in Lucknow to which reference has been made. The table sets forth the weight frequency distribution of male and female *M. rattus* respectively for each of the 12 months from February, 1911, to January, 1912. There appears to have been some influence at work destroying adult females and sparing the males. This "influence" began its manifestations in March and produced its maximum effect in May and June.

As if to compensate for the apparently wholesale destruction of adult females, females only appear to have been born. These two processes, the destruction of females and the suppression of male births, proceeded *pari passu.* In June not a single male rat below the weight of 80 grm. was trapped, whereas 610 females of less weight than 80 grm. were caught. As the numbers of adults of the two sexes began to approximate more closely the one to the other, young male rats were again trapped in increasing numbers. In November, December, and January, the sex ratio approximated to that normally pertaining.

Such, in brief, are the facts; satisfactory explanations of the phenomenon are difficult to come by. It may possibly be advanced that in the above brief recapitulation of observed facts I have assumed more than the facts warrant. The objection that is most likely to be raised is to the assumption that failure to catch adult females signifies destruction of females. The shyness of the female might account for the phenomenon. This point has been referred to, and it was partly to meet this objection that I studied similar facts concerning 95,629 rats caught in various places. A reference to the tables of Cawnpore, Banda, Coimbatore, and Ballia rats will show that in no place other than Lucknow was such a circumstance observed. Female rats are not shyer or more difficult to catch than males; on the contrary, it is possible that males are slightly more wary than females. As stated above the two sexes are normally produced in equal numbers, though adult females are usually in slight excess of adult males.

It may also be objected that the parallel assumption, that failure to catch young males signifies that no males are born, is not warranted by facts. This is admitted, but the only other explanation that I can offer is that the males were destroyed by their parents soon after birth (at a lesser weight than 10 grm., when it becomes just possible to trap them with the traps we employed, *i.e.*, at about a week old). That the parents should have destroyed only the male offspring is, to me, less easy of credence than that only females were produced.

It is a matter of regret that my observations did not succeed in throwing any light on the causes of the female mortality. Plague was certainly present until April, 1911, amongst the Lucknow rats, but it was not severe or widespread, and, as has been pointed out, plague is more fatal to the male than to the female. Whatever the cause was it was a widespread one in the city. The rats, caught from all parts of Lucknow, represented as fair a sample as could be obtained.

Further speculation on this interesting topic would not prove fruitful. The rapid readjustment of the sex ratio after so grave disturbance is, to my mind, the fact of chief interest. In May and June when hardly any females were produced there must have been an extreme degree of polyandry.

From a study of weight frequency curves of pregnant females, I have concluded that for practical purposes 90 grm. represents a fair dividing line between young and adult rats of Lucknow (Table VI). Half the rats of the weight of 90 grm. can be considered young and half adult. Employing this approximation, there appears to be an interesting correlation between the excess of adult males over adult females and the excess of young females over young males *for the same month.**

In spite of the absence of any explanation of the facts the phenomenon described appears to me to be of sufficient interest to warrant its publication.

* Mr. Major Greenwood, Jr., Statistician to the Lister Institute, has kindly supplied me with the approximate correlations between the sex ratios of mature and immature rats for the same month and also for certain sequences. The groupings appear in Table VII and the coefficients in Table VIII. It will be seen that the negative correlation for data derived from one month's records is slightly larger than when the records of successive months are combined. A possible explanation is that overlapping of different affected colonies produces an apparent synchronism of cause and effect, but the figures as they stand do not warrant any inference. The enormous excess of young females in May and June is a statistically inexplicable fact.

Table I.—Lucknow, 1911–12.

M. rattus.

Weight in grms.	February.		March.		April.		May.		June.		July.		August.		September.		October		November.		December.		January.	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0–20	53	73	44	56	4	122	0	83	0	108	34	85	34	110	46	118	102	114	80	93	8	17	41	30
21–30	54	33	52	103	8	123	0	100	0	169	31	153	65	186	109	220	134	180	178	185	67	71	45	85
–40	54	41	39	79	4	67	0	91	0	124	25	119	50	160	81	146	140	159	139	165	78	97	71	74
–50	37	41	35	57	2	47	0	85	0	99	24	107	37	114	75	144	135	135	121	122	70	78	64	76
–60	33	41	33	84	2	61	2	76	0	65	14	92	35	118	64	161	107	116	85	104	70	89	66	88
–70	43	60	28	53	1	51	0	62	0	65	22	79	37	132	63	124	113	116	103	108	60	102	67	74
–80	51	79	39	52	56	42	47	42	59	66	85	70	82	118	113	113	91	111	114	134	84	96	61	72
–90	34	54	37	48	29	48	36	38	48	51	56	73	75	81	103	123	72	92	88	125	62	94	73	90
–100	56	67	37	81	32	56	51	50	57	62	81	82	71	99	79	107	86	107	87	90	42	62	88	108
–110	52	89	50	85	36	70	42	55	44	40	70	76	53	94	60	120	67	89	61	89	52	77	86	84
–120	66	101	73	100	123	65	125	73	151	58	142	92	134	133	133	143	76	131	88	122	74	85	97	125
–130	87	136	145	174	164	130	216	99	204	88	201	118	214	153	211	171	130	158	108	150	91	121	100	150
–140	87	112	124	148	116	109	193	88	158	64	146	91	168	132	155	164	85	129	111	113	80	112	66	111
–150	72	78	132	108	140	98	183	93	150	63	155	91	141	97	150	106	77	87	61	70	60	65	77	79
–160	42	69	96	102	101	60	156	55	112	57	117	38	118	104	122	90	52	55	67	51	64	47	72	50
–170	69	69	111	71	119	48	108	50	97	32	115	48	125	67	110	89	54	74	91	44	65	44	64	52
–180	39	30	54	29	25	18	22	13	22	11	30	5	34	30	38	31	39	35	37	15	43	25	68	36
–190	29	17	46	25	32	15	21	11	16	8	21	11	32	20	36	24	31	22	28	13	33	6	39	15
–200	29	13	42	18	11	4	33	8	14	3	16	2	22	5	33	15	21	4	21	3	13	2	28	3
–210	19	3	24	4	7	3	9	2	7	2	14	2	18	4	15	6	9	7	11	1	12	3	19	2
–220	11	2	17	1	9	5	8	0	5	1	14	2	22	1	28	4	15	0	16	2	13	4	13	3
–230	9	—	14	—	12	—	4	2	4	1	3	—	7	1	11	0	7	1	8	1	1	1	9	2
–240	4	—	7	—	4	—	1	—	2	0	7	—	5	1	9	3	6	1	4	—	7	1	10	1
–250	3	1	8	1	9	—	7	—	1	1	2	—	9	2	4	—	1	0	3	—	0	1	5	0
–260	—	—	4	1	2	—	1	—	1	0	0	—	3	—	3	—	—	—	2	—	1	0	3	0
–270	—	—	—	—	1	—	0	—	—	—	0	—	1	—	1	—	—	—	2	—	0	0	0	0
–280	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1	—	—	—
Monthly totals	1033	1209	1291	1480	1049	1242	1265	1176	1152	1238	1426	1436	1592	1961	1852	2222	1651	1922	1714	1800	1151	1300	1336	1410
	2242		2771		2291		2441		2390		2862		3553		4074		3573		3514		2451		2746	

Grand total 34,908

Table II.—Cawnpore, 1911–12.

M. rattus.

Weight in grms.	March.		April.		May.		June.		July.		August.		September.		October.		November.		December.		January.		February.	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
11–20	4	2	5	12	36	24	40	40	58	46	62	34	20	18	20	14	9	5	2	1	—	—	16	4
21–30	56	39	40	27	107	64	108	85	109	78	120	90	94	57	79	66	42	41	16	20	21	13	45	32
–40	82	77	75	66	150	116	146	121	150	130	154	137	115	92	98	104	89	83	30	26	30	29	79	55
–50	132	126	106	83	219	194	235	221	261	190	197	229	226	185	251	204	203	169	79	68	43	56	95	81
–60	156	135	144	146	178	159	214	164	232	207	240	233	207	185	197	191	147	152	75	66	91	71	87	83
–70	90	119	131	102	166	131	189	160	174	187	227	198	221	207	233	203	193	209	104	108	82	77	78	59
–80	95	112	80	103	147	111	154	153	193	179	235	203	173	182	188	154	189	185	125	139	88	96	63	57
–90	47	63	78	75	94	92	130	140	159	200	174	229	130	174	159	197	182	155	122	132	86	118	82	93
–100	46	69	77	65	112	187	170	235	224	324	235	372	200	241	246	264	241	292	183	215	119	211	102	145
–110	67	138	96	157	87	141	111	175	167	248	194	323	144	206	167	191	135	201	111	141	179	244	72	130
–120	63	110	52	94	61	124	105	157	129	176	155	245	112	196	139	208	124	216	123	167	135	177	89	149
–130	56	148	37	60	58	105	114	159	140	163	180	226	80	157	124	176	126	161	112	110	122	193	92	135
–140	77	172	68	163	83	168	96	115	147	152	162	194	103	153	101	167	116	154	112	122	131	192	125	174
–150	77	155	82	192	92	115	93	129	120	128	150	143	102	119	114	129	102	109	142	106	195	201	136	120
–160	93	172	110	148	70	79	78	76	101	74	128	119	77	91	98	73	110	66	84	54	150	132	100	79
–170	80	128	79	111	67	59	74	44	71	49	107	70	76	60	80	53	74	42	95	34	131	100	76	62
–180	104	100	80	68	68	63	60	22	63	21	76	39	63	16	62	26	61	14	67	24	122	59	70	30
–190	47	35	62	27	50	25	45	12	53	18	70	15	53	20	68	14	56	14	42	9	89	25	69	33
–200	68	32	68	39	57	16	33	8	35	5	54	16	33	8	43	6	64	6	69	16	96	22	70	7
–210	47	22	64	18	35	8	16	1	21	2	38	2	21	4	21	6	17	0	38	3	51	9	32	3
–220	37	3	22	2	8	3	3	1	10	—	8	1	11	—	13	—	6	2	16	1	20	—	18	1
–230	22	4	15	2	3	—	1	1	3	—	7	1	1	—	6	—	4	—	9	1	17	1	4	—
–240	13	—	10	3	1	—	—	—	—	—	3	—	—	—	—	—	—	—	1	—	8	1	3	—
–250	3	—	2	0	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	4	—	1	—
–260	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
–270	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
–280	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
–290	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
Monthly totals	1563	1961	1583	1763	1949	1984	2215	2219	2620	2577	2977	3119	2262	2371	2509	2446	2290	2276	1758	1563	2013	2027	1604	1532
	3524		3346		3933		4434		5197		6096		4633		4955		4566		3321		4040		3136	
	Grand total … … … … 51,181																							

Table III.—Banda, 1911–12.

M. rattus.

Weight in grms.	November.		December.		January.		February.		March.		April.		May.		June.		July.		August.		September (half).	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
11–20	19	10	10	7	0	1	6	10	9	10	33	24	46	19	54	40	49	39	28	26	16	15
21–30	39	28	4	3	10	10	16	16	44	38	66	44	47	52	32	28	32	25	27	26	24	18
–40	28	33	6	11	6	7	16	18	30	28	59	38	34	18	17	26	35	19	12	27	18	23
–50	49	43	18	11	5	9	20	13	31	24	38	29	26	29	25	26	31	26	21	21	15	15
–60	50	59	17	20	17	11	8	13	20	18	29	24	27	22	21	21	32	16	25	20	24	11
–70	76	94	21	26	24	38	5	15	21	20	18	27	25	27	16	26	18	29	22	23	17	19
–80	88	102	35	48	28	49	10	27	20	39	15	18	27	29	16	20	32	42	24	36	27	24
–90	79	98	38	49	25	42	23	32	19	16	8	12	13	14	9	20	15	26	22	20	15	10
–100	92	109	45	58	44	68	39	65	31	40	14	41	10	19	13	16	25	47	19	36	12	24
–110	70	103	39	61	58	67	39	67	22	33	19	34	17	18	18	22	12	25	24	37	10	29
–120	90	102	47	64	44	98	55	86	40	59	22	48	9	20	10	24	22	33	20	31	11	12
–130	74	84	33	34	68	77	59	70	36	51	14	38	12	24	9	23	14	37	20	37	13	18
–140	50	62	33	34	49	33	56	31	33	30	14	17	10	10	9	6	16	15	13	25	13	12
–150	38	40	37	34	39	12	48	30	40	36	22	28	10	10	10	3	15	13	17	22	9	11
–160	24	19	10	9	30	18	38	12	31	22	20	12	8	4	5	1	8	12	13	13	9	5
–170	38	7	20	4	25	4	24	7	22	13	8	5	12	3	5	3	11	2	9	8	7	3
–180	24	4	10	2	17	1	18	5	15	7	11	4	4	2	2	3	7	1	6	2	8	0
–190	18	2	9	1	14	2	11	—	10	1	7	—	1	—	4	2	6	1	1	2	5	2
–200	8	1	9	—	25	3	17	—	9	—	3	—	1	—	3	—	11	1	9	—	3	—
–210	1	1	1	—	1	—	4	—	0	—	0	—	—	—	—	—	2	—	3	—	1	—
–220	1	—	2	—	—	—	0	—	3	—	2	—	—	—	—	—	—	—	2	—	—	—
–230	—	—	0	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
–240	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
–250	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
–260	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Monthly totals	956	1001	445	476	529	550	515	517	486	485	422	443	337	320	278	310	393	409	339	412	251	251
	1957		921		1079		1032		971		865		659		588		802		751		502	
Grand total 10,127																						

In May and June about 150 "adult" rats were sent to Bombay. In the above table, therefore, the relationship between the numbers of young and adult rats is misleading. The proportion of the two sexes, however, was not appreciably affected by the proceeding.

Table IV.—Coimbatore.

Weight in grms.	January to March.		April to June.		July to September.	
	♂	♀	♂	♀	♂	♀
11–20	29	27	33	44	30	35
21–30	49	47	78	86	119	114
–40	62	43	50	51	72	74
–50	45	45	66	49	87	64
–60	32	33	48	48	76	78
–70	15	31	30	34	72	71
–80	18	30	29	49	52	66
–90	14	30	33	60	58	72
–100	11	36	51	63	46	94
–110	13	23	21	57	33	80
–120	14	32	31	59	52	83
–130	18	16	21	29	40	56
–140	9	11	23	15	37	38
–150	11	7	16	14	20	27
–160	6	2	18	11	28	9
–170	6	2	15	7	27	10
–180	6	0	9	0	15	5
–190	1	1	4	1	9	1
–200	1		1	1	3	1
–210	1				2	
–220	1				0	
–230						
–240						
–250						
Totals ……	362	416	577	678	878	978
	778		1255		1856	

Table V.—Ballia.—Consecutive rats caught between January 23 and October 6, 1911.

M. rattus.

Weight in grms.	♂	♀	Weight in grms.	♂	♀
11–20	102	82	160	74	142
21–30	182	171	170	67	89
–40	147	139	180	49	51
–50	127	135	190	51	34
–60	98	116	200	58	23
–70	127	109	210	34	9
–80	110	113	220	21	1
–90	100	134	230	4	2
–100	127	155	240	1	0
–110	89	168	250	0	0
–120	128	222	260	0	0
–130	102	248	270	0	0
–140	103	224	280	0	1
–150	74	182			

Table VI.—Lucknow.

M. rattus.

Weight in grms.	Number pregnant.	Number of females.	Per cent.	Number of fœtuses.	Average.
51–60	6	1097	0·5	31	5·1
61–70	29	1026	2·8	135	4·6
–80	70	995	7	371	5·3
–90	120	917	13	614	5·1
–100	151	971	15·5	772	5·1
–110	200	968	20·6	1135	5·6
–120	303	1208	25	1670	5·5
–130	429	1648	26	2444	5·7
–140	423	1373	30·8	2509	5·9
–150	359	1034	34·7	2154	6
–160	292	778	37·5	1795	6·1
–170	305	678	44·8	2000	6·5
–180	134	279	48	856	6·4
–190	101	187	54	657	6·5
–200	40	80	50	272	6·8
–210	21	39	53·8	118	5·6
above 210	32	48	66·6	217	6·8

Table VII.

Weight in grms.	♂	♀	Total.
	April.		
90 or less	106	561	667
Beyond 90	943	681	1624
	1049	1242	2291
	May.		
90 or less	85	577	662
Beyond 90	1180	599	1779
	1265	1176	2441
	March.		
90 or less	307	532	839
Beyond 90	984	948	1932
	1291	1480	2771

Weight in grms.	♂	♀	Total.
	May–April.		
90 or less	85	577	662
Beyond 90	943	681	1624
	1028	1252	2286
	May–March.		
90 or less	85	577	662
Beyond 90	984	948	1932
	1069	1525	2594
	April–March.		
90 or less	106	561	667
Beyond 90	984	948	1932
	1090	1509	2599

Table VIII.

Source of Mature Rats (over 90 grm.).	Source of Immature Rats (90 grms. or less).	Coefficient of Correlation.*
March data	March data	$-0 \cdot 23 \pm 0 \cdot 04$
April „	April „	$-0 \cdot 70 \pm 0 \cdot 02$
May „	May „	$-0 \cdot 81 \pm 0 \cdot 01$
April „	May „	$-0 \cdot 76 \pm 0 \cdot 02$
March „	May „	$-0 \cdot 69 \pm 0 \cdot 02$
March „	April „	$-0 \cdot 62 \pm 0 \cdot 02$

* The coefficients were determined by means of Pearson's approximate method ('Phil. Trans.,' A, vol. 195, p. 16, equation lvii) and the probable error assumed to be $3[0 \cdot 67449(1-r^2)/\sqrt{n}]$.

The Conduction of the Pulse Wave and the Measurement of Arterial Pressure.

By Leonard Hill, F.R.S., James McQueen, and Martin Flack.*

(Received December 20, 1913,—Read February 5, 1914.)

(From the Physiological Laboratory, London Hospital Medical College, London Hospital Research Fund; and the Pathological Laboratory, Aberdeen University.)

It is now well established that in cases of aortic regurgitation placed in the horizontal position the arterial pressure is considerably higher (50–80 mm. Hg) in the leg than in the arm.† Such pressures are taken by the sphygmomanometer, using the armlet method, the armlet being placed respectively round the calf and the upper arm, the disappearance and reappearance of the pulse wave being noted in the dorsalis pedis or posterior tibial artery, and in the radial.

In seeking for an explanation of this phenomenon it has already been suggested by us‡ that the "lability" of the arterial wall plays a considerable part, the term "lability" being used to designate the ease with which an artery distends with a rise and recoils with a fall of arterial pressure. The effect of increased and of diminished lability of the vessel wall upon the conduction of the pulse wave has been demonstrated schematically by

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* During tenure of Eliza Ann Alston Research Scholarship.

† Hill, Flack, and Holtzmann, 'Heart,' vol. 1, p. 73 (1909); also Hill and Rowlands, 'Heart,' vol. 3, p. 222 (1912).

‡ Hill and Flack, 'Roy. Soc. Proc.,' B, vol. 86, p. 365.

Russell Wells* on rubber tubing made with a thickness of wall corresponding to an artery. While the lability effect has been shown by us in exposed arteries, in the body the main arteries are surrounded with tissues permeated with small arteries into which the blood pulsates. As the arterial wall is supported by the pulsing tissues the lability effects obtained on the exposed arteries cannot be directly ascribed to the same arteries *in situ.* Further investigation must be made on these.

Now J. McQueen, Ingram, and Leonard Hill† have shown that there is an extraordinary difference in the pressure required to damp down the pulse wave in arteries such as the aberrant radial and the dorsalis pedis, where these run superficially and lie upon bone, as compared with the same or other arteries lying in the midst of pulsating "resonating" tissues. They suggest that the pulse wave is supported on its way to the periphery by the pulsing tissues, and that the higher leg readings obtained in cases of aortic regurgitation may be due in part to the better conduction of waves which have a high crest through the pulsating mass of the abdomen and thigh. The reading of pressure in the case of the aberrant radial or dorsalis is taken with the Leonard Hill pocket sphygmometer. The small bag of this instrument when pressed on the radial artery (embedded in the tissues of the forearm) gives the same readings as the armlet method. When pressed on the aberrant radial or on the dorsalis pedis a far lower reading is obtained, *e.g.*, in a youth 35 mm. Hg against 110 mm. Hg.

We have constructed a wooden C-shaped box in which the arm can be suspended freely by a sling. If the armlet be placed round this box so that it presses on the front of the forearm, the obliteration of the pulse in the radial is obtained by the same pressure as is required if the armlet be used in the ordinary way. If the forearm be put in the box with the radial border uppermost, and the aberrant radial be pressed upon by the armlet, then the pulse is obliterated by a pressure of 35 mm. Hg. Using the armlet in the usual way the pulse is obliterated in this artery by 110 mm. Hg. In the one case the artery lying on bone is deformed by the armlet just as it is deformed by the bag. In the other case the pulse in the aberrant radial is not obliterated until the systolic pressure in the tissues of the forearm is overcome.

We have recently investigated several cases of "high blood-pressure" and find the following divergence between the readings of the leg and arm arteries, using the armlet and the dorsalis pedis, the patients being in the horizontal posture :—

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* Russell Wells and Leonard Hill, 'Roy. Soc. Proc.,' B, vol. 86, p. 180.

† J. McQueen, Ingram, and Leonard Hill, 'Roy. Soc. Proc.,' B, vol. 87, p. 255 (1913).

	Arm, armlet.	Leg, armlet.	Dorsalis, L.H. small instrument.	Remarks.
	mm. Hg.	mm. Hg.	mm. Hg.	
G	225	275	139	Myocardial failure.
G′	250	295	130 (Temporal 135)	„ „ (aortic regurgitation found *post mortem*).
R	215	260	130	Myocardial failure.
C	185	255	100	„ „
H	130	215	45	Aortic regurgitation.
M1	180	175	65	Chronic nephritis.
M2	170	175	55	„ „
C1	105	175	80	Mesaortitis.

In a normal individual we have found also that the variation in pressure found on changing from the horizontal to the vertical position fully accords with the effect of gravity, and that this is so when the readings are taken from the dorsalis pedis or with the armlet round the leg. The dorsalis reading in the vertical posture is increased by the gravity pressure just as much as is the leg reading.

The divergence in readings between the artery lying in tissues or exposed lying on bone has been fully substantiated by us in animals. If we place round the neck of a dog an armlet connected with a recording manometer and at the same time record the blood-pressure in the carotid artery by a v. Basch C-spring manometer, we find that the pressure required to obliterate the pulse wave on the tracing of the C-spring is just about the same as the actual systolic blood-pressure. To graduate the C-spring we

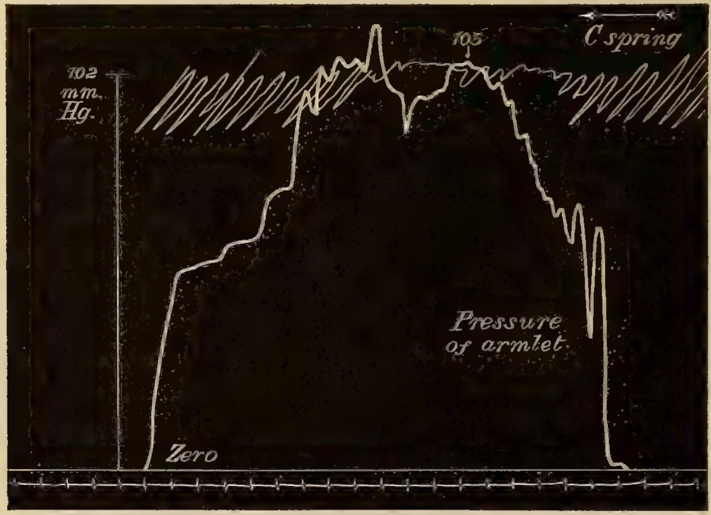

Fig. 1.

connect it with the Hg manometer and force up the pressure till the writer of the C-spring reaches the crest of the pulse curve (fig. 1).

We obtain similar results on applying to the carotid artery (*in situ*) of the cat the bag of the small Leonard Hill instrument instead of the armlet.

On the other hand, if we place a long length of the exposed carotid artery on the convex surface of a watch-glass, we find that the pulse wave is obliterated by a pressure in the bag much less than before. For example, in a dog with an arterial pressure of 190 mm. Hg. it was found that with the armlet 190 mm. pressure was required; in the same animal with the artery exposed but 60 mm. Hg. was required. In a cat we found—

The systolic pressure was...............	65	mm. Hg.		
And with the artery unexposed	64	„	was necessary	to damp down pulse.
Artery lying exposed on muscles	26	„	„	„
Artery lying on scalpel handle (*cf.* fig. 2B).	12	„	„	„

To elucidate the cause of this marked difference in readings we devised the following experiment:—A long length of cat's carotid was exposed, the uppermost part ligated and divided. This end was first passed through a T-piece and then an arterial cannula inserted into it, which in its turn was connected with the C-spring manometer. As the artery passed in and out of the T-tube through a piece of rubber tubing, the latter could be constricted

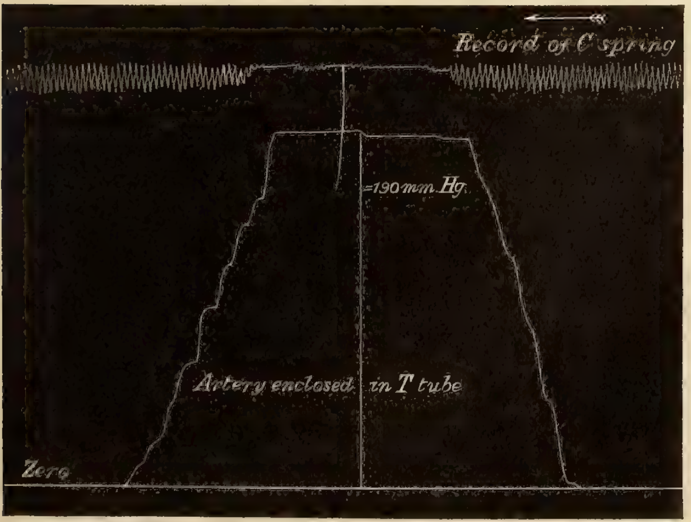

Fig. 2A.

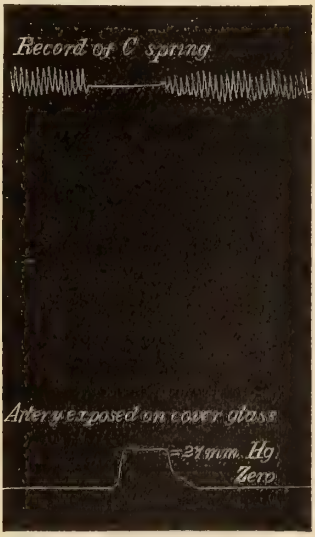

Fig. 2B.

so as to prevent any considerable leak of fluid from the T-tube. The artery in the T-tube was then surrounded by Ringer's solution and the pressure of this raised. Obliteration of the pulse wave occurred when the pressure in the T-tube reached that in the artery—190 mm. Hg. (fig. 2A). The same exposed artery removed from the T-tube and placed across the dome of a watch-glass required but 27 mm. Hg (fig 2B).

This experiment demonstrates the fact that the deformation of the arterial lumen is the prime cause of the obliteration of the pulse wave. When the artery is circularly and equally compressed by surrounding fluid, the pulse continues to come through until the full systolic pressure is overcome. In the case of the artery exposed and lying on a rigid surface, when the bag is pressed on it the arterial wall is pushed in above and bulged out at the sides. The lumen is thus converted from a circle into an ellipse, and resistance is offered to the pulse by the changed shape. The force of the pulse is spent on the labile wall of the artery in front of this resistance.

In corroboration of this experiment we have also found that if the finger be gently laid along the course of the radial artery and the bag of the sphygmomanometer pressed upon the finger until the pulse ceases to come through under it, a less pressure is required than without such interposition of the finger. This is because the finger brings about the deformation of the artery more easily than the bag. If the armlet be used and the finger be inserted under the armlet to palpate the artery, one finds that the pulse does not cease to come through under the finger until the full systolic pressure is reached. Thus the readings were 35 and 97 mm. Hg respectively in the case of a youth.

In the aberrant radial artery the pulse was obliterated by 55–60 using the bag, by 35 using finger and bag. Using armlet and finger the radial was obliterated by 135 and using armlet alone by 135.

A thin, distensible rubber bag inflated with a pressure of air can easily be deformed from the spherical to elliptical or other shape without altering the internal pressure. The bag may thus be made to take a shape which would give great resistance to the passage of a pulse wave or flow of fluid, although the total volume and pressure of the air in the bag remains unaltered.

In the experiments on animals (goat, dog, cat) it was found that the pressure required to obliterate the pulse wave in the exposed artery varied from 25 to 60 mm. Hg. To elucidate the cause of the higher and lower readings we measured the pressure necessary for the obliteration of the pulse in the same exposed artery with the animal (cat) in the head-down, horizontal, and feet-down position. We found that the pressure necessary to obliterate the pulse varied markedly with the diastolic pressure within the artery:—

	Diastolic pressure.	Obliteration pressure.
	mm. Hg.	mm. Hg.
Head-down	160	48
Horizontal	134	28
Feet-down	125	20

The lability of the wall also plays a part, since with the same diastolic pressure a higher pressure is required to obliterate the pulse in the carotid artery of the dog or goat than in that of the cat.

McQueen, Ingram, and Leonard Hill found that when the pulse in the aberrant radial artery was obliterated by a pressure in the bag of, say, 45 mm. Hg, the blood still trickled slowly into the artery. We have made a cut into the exposed carotid of a cat and found that a pressure of 26 mm. Hg stopped the visible flow; a pressure of 20 mm. Hg allowed slow oozing from a very elliptical lumen; while a pressure of 10 mm. Hg allowed the blood to spout freely through the incision.

In the above investigations the remarkable fact comes out that the pressure required to deform the artery and to obliterate the pulse wave is considerably below even the internal diastolic pressure of the vessel. To investigate this phenomenon further, we compared the effect of perfusing, with the same pulsating head of pressure, thin rubber tubing (about 0·7 mm. thick) and a length of human artery of approximately the same calibre and thickness of wall, and noting the external pressure required to obliterate the pulse wave The results are as follows:—

	Systolic pressure.	Obliteration of pulse, pressure in bag.
	mm. Hg.	mm. Hg.
Rubber	140	195 (fig. 3)
Artery	130	46 (fig. 4)

The rubber tube resisted deformation, the labile arterial wall suffered deformation easily.

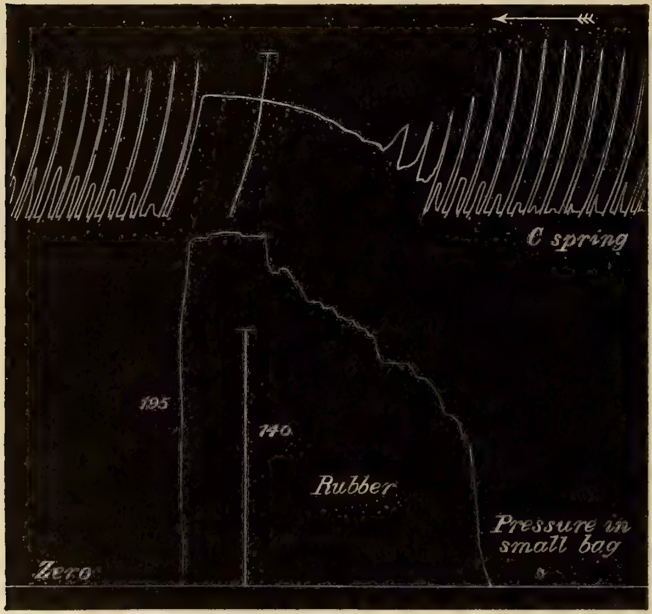

FIG. 3.

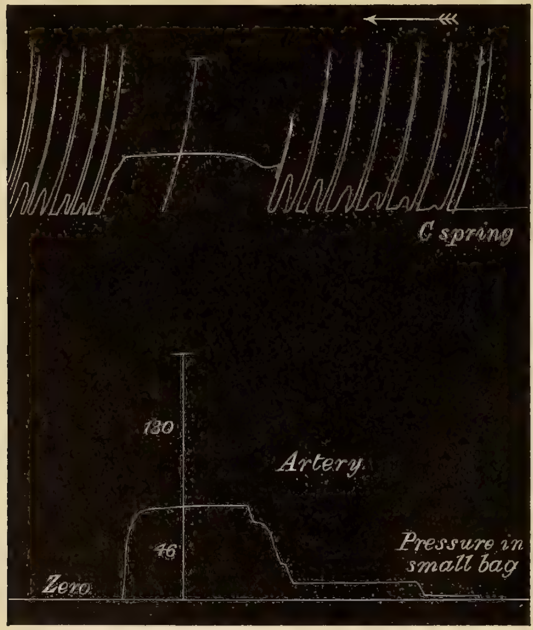

FIG. 4.

We then immersed a piece of the same rubber tubing in xylol. The tubing quickly imbibed xylol and became swollen, less elastic, and more easily distended or deformed. Experimenting in the same manner with this xylol-soaked tube we obtained—

Systolic pressure.	Obliteration pressure.
mm. Hg. 133	mm. Hg. 95 (fig. 5)

Thus the rubber tube by soaking in xylol was brought to resemble the artery.

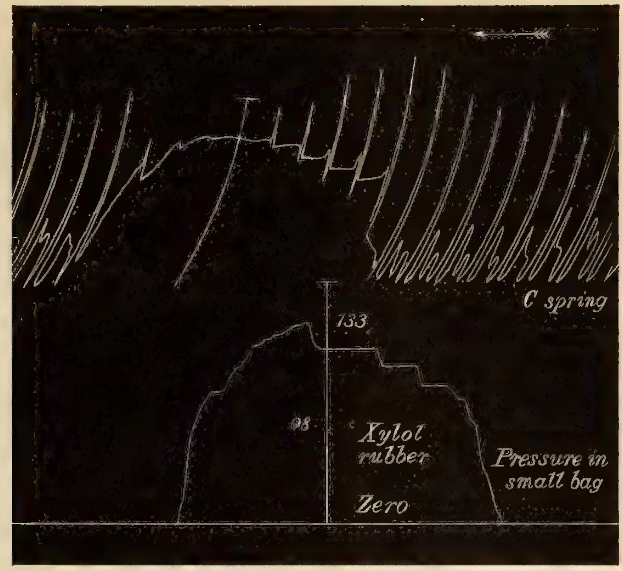

Fig. 5.

Further experiments we have done on human arteries are these:—

I. We bandage the arm with a rubber bandage, place the armlet on the upper arm, raise the pressure in it above systolic pressure, and then remove the bandage—the arm is left exsanguined. We now place the sphygmograph (using the weight extension method) in position on the radial artery, then let go the armlet. We find that the pulse curve returns slowly to its full amplitude when the weight extension is 300 grm., while it returns almost instantaneously when the weight is 150 grm. (see fig. 6, 2 and 3). When the heavier weight is used the pulse wave does not lift it until the tissues fill with blood and the peripheral resistance increases to such a degree that the systolic pressure in the surrounding tissues and artery overcomes the pressure of the sphygmograph pad which is pressing upon them. If the arm be not exsanguined the pulse returns at once to its full amplitude in spite of the heavier extension weight (fig. 6, 1).

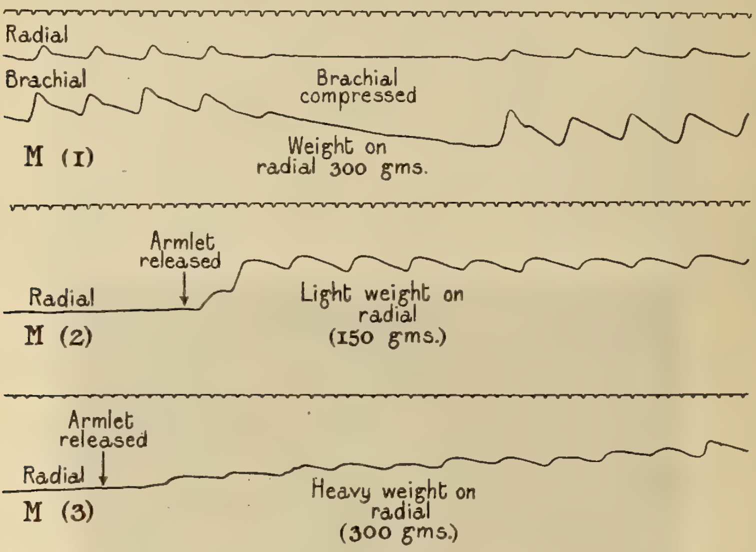

Fig. 6.

II. We surround the upper arm with ice, while the lower arm is immersed in hot water. After a few minutes the obliteration pressure is taken with the armlet on the flushed forearm, while the upper arm is still encased in ice. Under these circumstances we have generally found that the pressure in the lower arm is 15–20 mm. Hg higher than it was before the experiment. The ice is then removed from the upper arm and the pressure quickly taken with the armlet there. In this case the pressure is generally slightly lower

	Before experiment, armlet round either upper arm or forearm.	Armlet round flushed forearm, upper arm cold.	Armlet round upper arm, ice just removed.
	mm. Hg.	mm. Hg.	mm. Hg.
G. S.	97	120	95
G. S.	97–100	120	95
J. McQ.	130	142	125
M. F.	115	125	112
M. F.	105	105	85–88
	—	95 (Other arm, 105)	90–95 (15 minutes later)
S. E.	105–108	115	103–105
		—	97 (5 minutes later)
		105 After application of ice to forearm	

(3–5 mm. Hg) than at the beginning of the experiment. In one case, when the application of the ice had been so long that the skin of the arm had become red in patches, the pressure in the upper arm was lowered 20 mm. Hg.

It is a remarkable fact that the readings obtained from the cold upper arm should be lower than those obtained from the flushed forearm.

The tentative explanation we offer of these results is as follows. The vessels in the tissues of the upper arm under the influence of the ice are constricted and largely exsanguined, thus the artery is less well supported by the resonance of the pulse in these tissues, and is therefore deformed by a lower pressure than is required in the flushed forearm, where the resonance of the systolic wave is greater. At the same time the cold contracted artery in the forearm conducts the crest of the wave better to the flushed forearm since it is less labile. The readings obtained from the cold upper arm show that the wall of the artery, even though contracted by cold, does not offer such a resistance to compression as to influence the readings. We (L. H. and M. F.) reached the same conclusion by methods we devised for testing the readings we obtained in cases of high blood-pressure.*

Conclusions.

We conclude that the armlet or Leonard Hill's small bag, applied to the radial artery, give, under ordinary conditions, accurate readings of systolic pressure, the obliteration of the pulse being taken as the index. This is because the artery is surrounded by pulsing tissues and cannot be deformed until the systolic pressure is overcome in these tissues. The artery is equally compressed on all sides by a pulsating fluid pressure and the conditions are the same as when it is compressed in a glass T filled with Ringer's fluid. In the dorsalis pedis, the temporal, or aberrant radial artery, where lying on bone and tendon, the pulse is obliterated by a pressure of the small bag much lower than the systolic pressure. This is because the lumen of the labile arterial wall is deformed more easily under these conditions from the circular to an elliptical shape, and the resistance to the passage of the pulse wave thereby increased.

The higher the diastolic pressure the greater must be the pressure of the bag to produce the required deformation. As the amplitude of the pulse wave depends so much on the size of the lumen, it seems probable that the higher readings obtained in cases of aortic regurgitation are due in part to the lumen of the aorta, iliac and femoral arteries being relatively wider than that of the subclavian and brachial arteries. The pulsating (resonating) support given to the former arteries by the relatively massive abdominal organs and the tissues of the thigh may also help to prevent the damping of the crest of the pulse waves. The leg arteries are probably held in a more supported state, less labile, and for this reason also the pulse will be conducted to the leg with less diminution in force. Size of lumen, resonance and lability are three factors which may all take a part in the production of this phenomenon. We have brought forward in this paper experiments which demonstrate these factors at work.

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On the Floral Mechanism of Welwitschia mirabilis, *Hooker.*

By Arthur Harry Church, Lecturer in Botany, University of Oxford.

(Communicated by Prof. A. C. Seward, F.R.S. Received December 23, 1913,—Read February 5, 1914.)

(Abstract.)

1. In the preparation of sectional schemes for the flowers of *Welwitschia mirabilis,* in different stages of development, several points of interest were noted as tending to throw light on the previous history of this unique floral form.

2. Evidence is adduced to show that the primary structural features of the flowers are referable to an anthostrobiloid condition closely comparable with that of *Cycadeoidea,* now expressed in a phase of minimum reduction, and to be regarded as an example of heterophyletic convergence to a simple floral construction in the gymnospermic condition.

3. Secondary features of biological interest are mainly consequences of xerophytic specialisation in the inflorescence; condensation of the whole system to a "cone" necessitates the extreme flattening of the flower in the transverse plane, which has led to confusion in the interpretation of the facts of development; the andrœcium is represented by a true whorl of six members.

4. Similarly, secondary clisanthy in the cone mechanism necessitates special features in the individual flowers, and accounts for the long exserted micropylar tube of the ovulate flower, and the protrusion mechanism of the staminal tube in the sterile flower.

5. The working mechanism of the latter is clearly indicated by remarkable phenomena of the storage and subsequent disappearance of starch in the androecial region; while similar phenomena of starch storage and depletion in the gynoecial region illustrate the progression from a simple "drop-mechanism" to a copious exudation of sugar and the adaptation of the structure to entomophily.

6. The nectary region of the ovule is retained by the gynoecium of the sterile flower for the same function, and vascular bundles supplying fluid for this purpose are not necessarily vestigial. Entomophily is thus probably antecedent to dicliny.

The Influence of the Position of the Cut upon Regeneration in Gunda ulvæ.

By Dorothy Jordan Lloyd, B.Sc., Bathurst Student of Newnham College, Cambridge.

(Communicated by J. Stanley Gardiner, F.R.S. Received January 14,—Read February 19, 1914.)

I. Introduction.

In 1899 Hallez (4) made the generalisation that the most important difference between the regeneration in Triclad and Polyclad Planarians was to be found in the fact that fragments of the former could regenerate in the absence of the central nervous system, whilst in the latter some portion of the cerebral ganglia must be present in order for regeneration to take place. Child (1) has confirmed the fact that the presence of cerebral ganglia, or at least intact nerve roots, is necessary for regeneration of the anterior end and sense organs of Polyclads. The experimental work by the same and other authors has also established that, among Triclads, the genus *Planaria* is able to regenerate completely in the absence of cerebral ganglia. The following notes, however, show that in another Triclad genus, namely, *Gunda*, anterior regeneration is, as in Polyclads, dependent on the presence of the central nervous system.

The experiments described below were carried out in the Plymouth Laboratory of the Marine Biological Association during the spring of 1913. I am greatly indebted to the director and staff of the laboratory for constant kindness during the course of my work at Plymouth. I also stand under obligations to the Royal Society, the Zoological Society, and the University of Cambridge for the use of their tables at the Plymouth Laboratory.

II. MATERIAL.

The experiments were performed on members of the species *Gunda ulvæ*. *G. ulvæ* is a small Triclad Planarian belonging, with other marine planaria, to the order Maricola, and to the family Gundidæ (Procerodidæ). *Planaria*, the genus on which most of the work on Triclads has been done, is a fresh-water form (order Paludicola).

The structure of *G. ulvæ* is quite typical of all Triclads and has been described by Wendt (8) and Iijima (5). It contains the usual trifid gut, which opens at the point where the three branches meet into a protrusible pharynx. The mouth is situated near the hind end, rather more than three-fourths down the length of the body. The accessory genital organs lie behind the mouth. The cerebral ganglia are about one-fifth the body length from the anterior end. Two nerve cords run backwards from the ganglia, joining in the tail to form a complete ring. The two eyes lie in front of the ganglia, and at the front of the head are the so-called auricular processes, two in number.

III. EXPERIMENTS.

A. *Posterior Regeneration.*

G. ulvæ will regenerate tails completely either in the presence or absence of the cerebral ganglia. Text-fig. I, A, shows the levels at which cuts were made. Fragments taken for posterior regeneration were head-pieces ADD, AEE, AFF; middle pieces, BBFF and DDFF. Figs. 1–3 show the progress of tail regeneration in a worm cut through the middle of the pharynx region (AFF). For worms cut off in front of the pharynx (AEE, I, 4), it is essentially similar, except that the newly regulated worm is proportionally smaller. The figures, which are drawn to scale, show quite clearly that the new tail is produced by a differentiation of the old tissue.

The next group of text-figures shows the regulation of fragments taken from the middle of the worm. The characteristic difference in behaviour between the pieces with and without cerebral ganglia (figs. 5–7 and 8–10 respectively) is quite apparent. The former show regeneration taking place at both ends of the worm, finally resulting in complete restoration of both head and tail. The formation of tissue at the head end is seen to check the rate of growth at the tail end. In fragments without ganglia (figs. 8–10) no head is regenerated and restoration of the tail proceeds as rapidly as in the head-pieces (with ganglia) described first. Restoration of posterior parts takes place at any aboral surface from DD backwards and is independent of the presence of the ganglia. Fragment ABB, or fragments equally small taken from any part of the body, die without any regeneration.

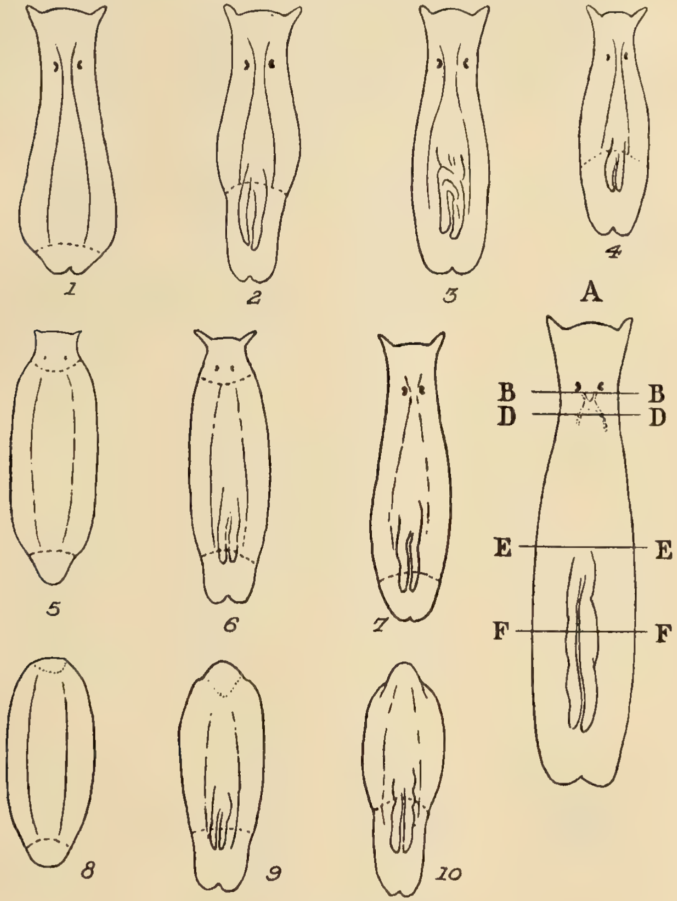

Fig. I.

1. Fragment AFF, 14 days; 2. Ditto, 28 days; 3. Ditto, 56 days. 4. Fragment AEE, 32 days. 5. Fragment BBFF, 14 days; 6. Ditto, 28 days; 7. Ditto, 56 days. 8. Fragment DDFF, 14 days; 9. Ditto, 28 days; 10. Ditto, 56 days. A. Diagram of points of section.

B. *Lateral Regeneration.*

Animals bisected down the median line (text-fig. II) regenerate completely. The course of regeneration is shown in figs. 1–3.

In animals beheaded and then bisected down the middle line (fragment PD), regeneration occurs along the edge of the lateral wound, but there is no replacement of the anterior end (text-fig. II, 8–11) unless parts of the

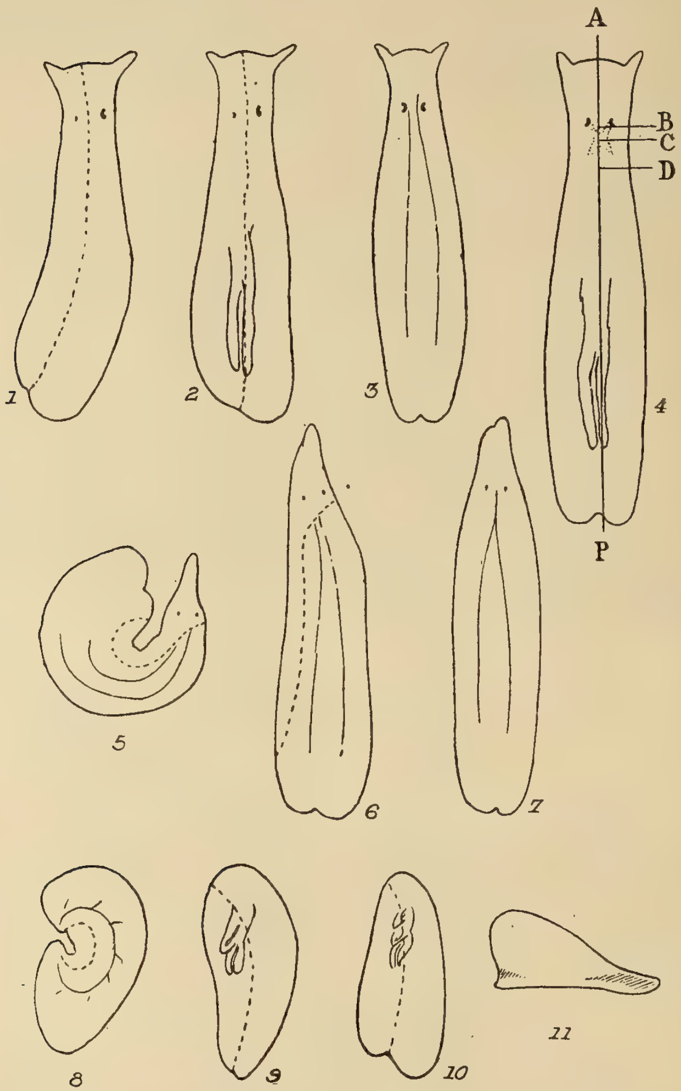

Fig. II.

1. Fragment AP, 20 days; 2. Ditto, 27 days; 3. Ditto, 41 days. 4. Diagram of points of section. 5. Fragment CP, 20 days; 6. Ditto, 41 days; 7. Ditto, 62 days. 8. Fragment DP, 20 days; 9. Ditto, 32 days; 10. Ditto, 41 and 62 days. 11. Same, side view.

cerebral ganglia are left in the fragment. In this case there is some regeneration of tissue at the anterior end, but the heads produced are defective (text-fig. II, 5–7).

C. *Anterior Regeneration.*

Anterior regeneration in animals cut anteriorly to the cerebral ganglia is quite complete (text-fig. III, 1–3). In cases, however, where the cut takes away more than about one-third of the ganglia, it is only partial. Forms with either two eyes or one are produced, the auricles in the latter case being fused (figs. 4 and 5). In animals cut behind the cerebral ganglia no head formation ever occurs. These posterior pieces showed some change of form, as forward growth caused them to become helmet shaped (fig. 6). After two months these pieces still remained without any attempt at regeneration of the head.

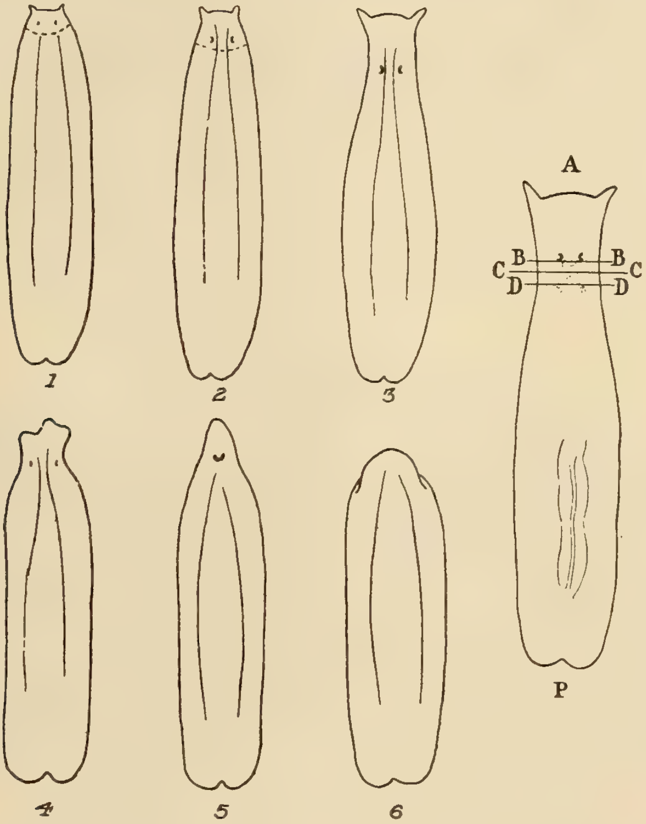

Fig. III.

1. Fragment BBP, 14 days; 2. Ditto, 28 days; 3. Ditto, 41 days. 4. Fragment CCP, 41 and 62 days. 5. Fragment CCP, 41 and 62 days. 6. Fragment DDP, 41 and 62 days.

A number of headless pieces and defective heads were sectioned for examination of the nervous system, and the sections are shown.

It is obvious that in cases of incomplete regulation the central nervous system is incomplete in corresponding extent.

D. *Heteromorphic Forms.*

The culture which produced these forms was one of very short head-pieces where the cut had passed across the anterior part of the brain. The tail-pieces corresponding to these head-pieces also produced new heads. The heteromorphic forms 20 days after section are shown in fig. IV. In cases where the cut passed obliquely the new heads formed on the anterior end of the cut. This is shown in fig. IV (2).

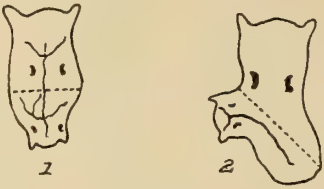

Fig. IV.

1 and 2. Fragment ACC, 20 days.

A longitudinal section through a heteromorphic form is shown in fig. IX. The rounded shape of the section is due to the contraction that takes place when the animals are dropped into the fixing fluid. It can be seen in the section that about one-third of the complete brain is present. Two well-marked nerves run to the new eyes, and the portion of regenerated gut in the new head shows a beginning of the formation of the three branches characteristic of the front end of the gut of *G. ulvæ*. The gut is full of a dark brown mass of broken-down tissue, and can be seen clearly in the whole specimens.

E. *Regeneration of Nervous System.*

In planarians, *i.e.*, in *P. torva* (Flexner (3) and Schultz (7)) and *Planaria* (*Dendrocœlum*) *lactea* (Schultz (7)) the new nervous system arises by cells from the parenchyma crowding round the cut ends of the nerve cords and pushing up among the old nerve fibres, aided possibly by some outgrowth of the old fibres. This is also the method by which *G. ulvæ* repairs injuries to the nervous system. The nerve cords of *G. ulvæ* always show regeneration after a cut, and in every case of tail-formation the new nerve fibres can be found reaching to the end of the newly formed tail at every stage of its growth, and within a few days of the cut joining at the hind end to form the posterior commissure.

After a longitudinal bisection the brain will easily regenerate the removed half, and the circuit of the nervous system is restored, often before the long wound has healed over. Transverse sections across the ganglia, however, are not followed by regeneration of the ganglia if more than about one-third of the brain is taken away. The nervous system forms a complete ring, and in every case, where this ring is broken, regeneration of the nerve cords takes place sufficiently to restore the circuit, though the cerebral ganglia may or may not be regenerated.

Text-fig. V shows an animal (text-fig. III, 3) where the cut passed across the front of the ganglia, which were subsequently completely restored. Text-Fig. VIII (text-fig. III, 6) shows an animal in which the cut removed both ganglia. In such conditions the nerve cords grow forward and fuse, but no ganglia are regenerated, and the animals remain headless. Text-figs. VI and VII show two worms (text-fig. III, 4 and 5 respectively) where the cut has damaged both ganglia and where there is correspondingly defective restoration of the head.

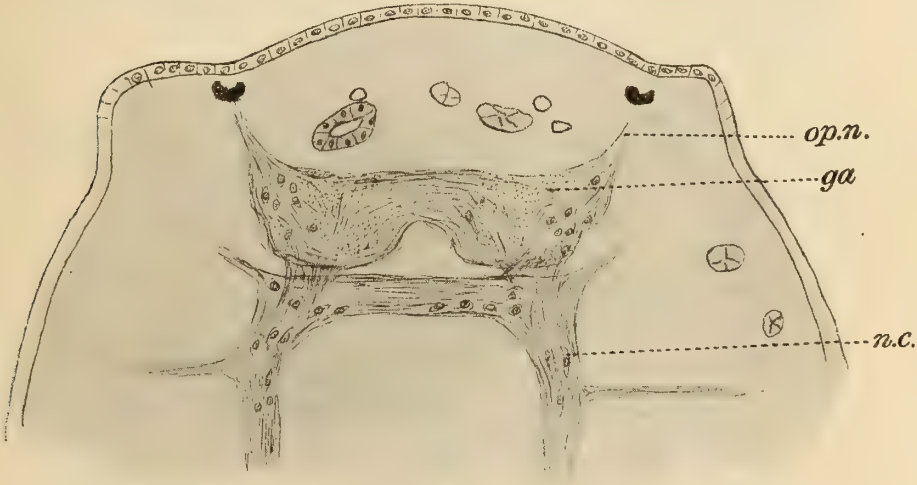

FIG. V.—Horizontal Longitudinal Section through Complete Regenerated Head, 41 days (text-fig. III, 3). *ga.*, ganglion ; *n.c.*, nerve cord ; *op.n.*, optic nerve.

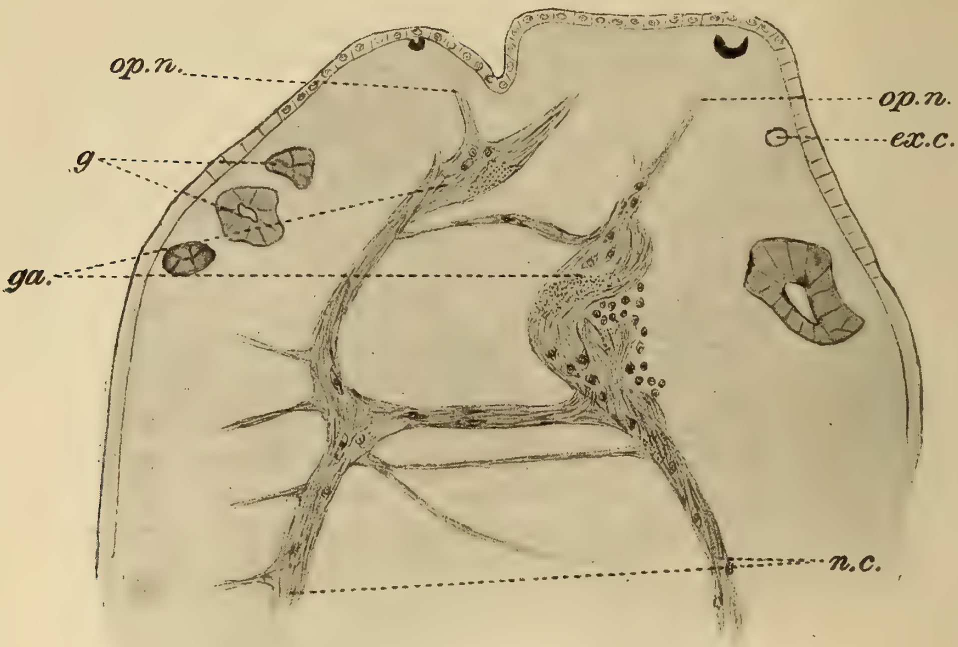

FIG. VI.—Section through Incomplete Regenerated Head, with two eyes, 62 days (text-fig. III, 4). *ex.c.*, excretory canal ; *ga.*, ganglion ; *g.*, gut ; *n.c.*, nerve cord ; *op.n.*, optic nerve.

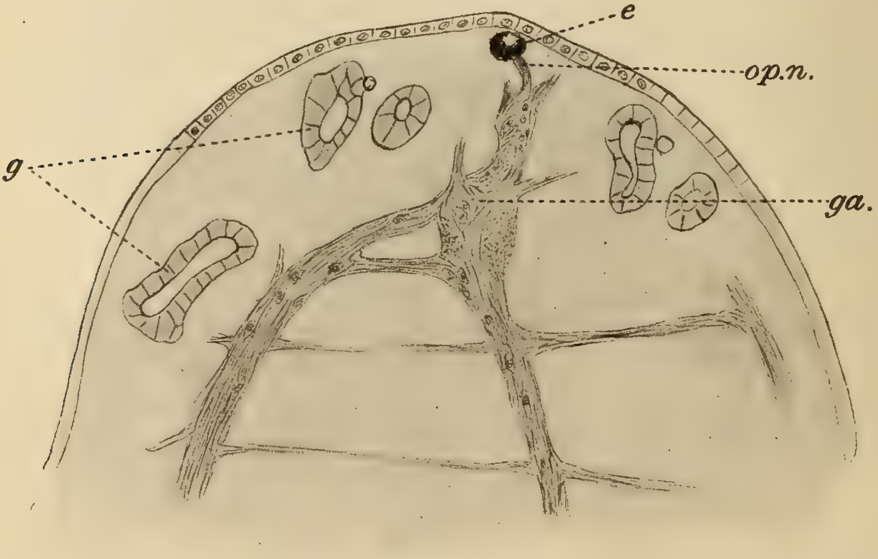

FIG. VII.—Section through Incomplete Regenerated Head, with one eye, 64 days (text-fig. III, 5). *e.*, eye ; *g.*, gut ; *ga.*, ganglion ; *op.n.*, optic nerve.

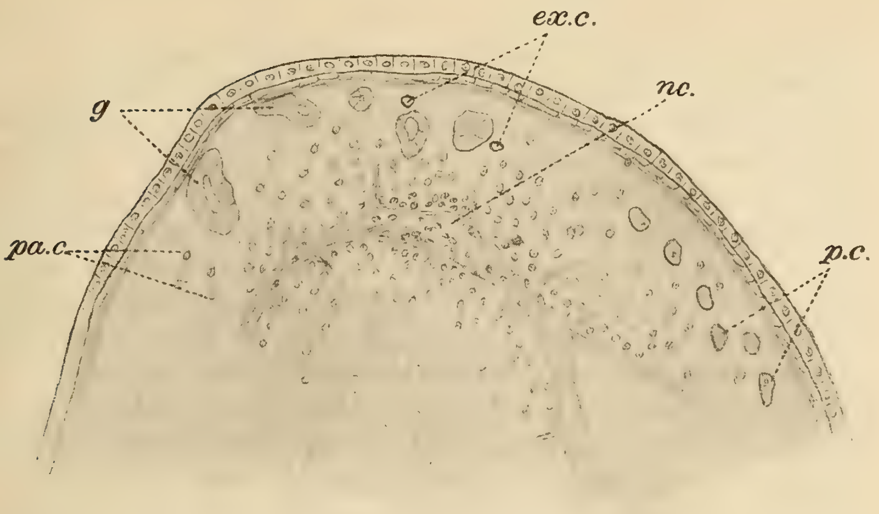

Fig. VIII.—Section through Headless Form, 62 days (text-fig. III, 6). *ex.c.*, excretory canals; *g.*, gut; *n.c.*, nerve cords; *pa.c.*, parenchyma cells; *p.c.*, pigment cells.

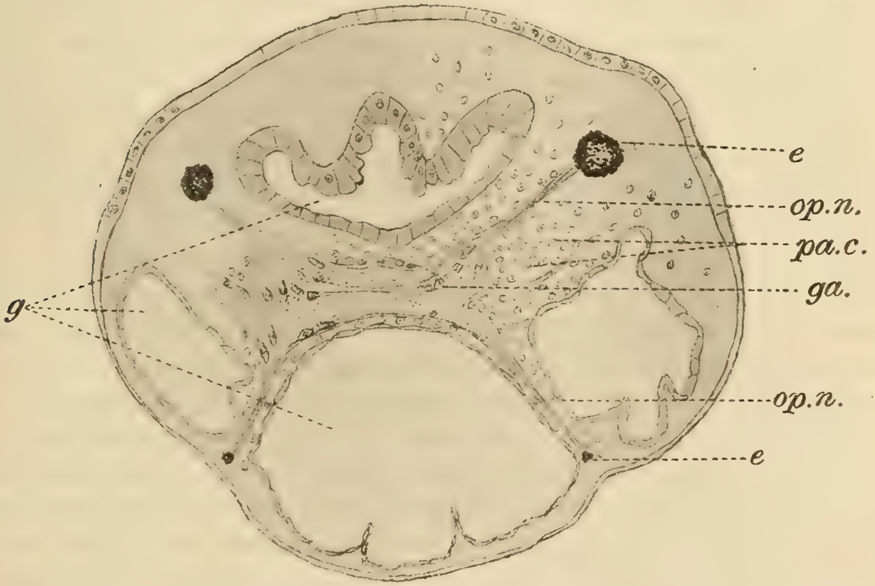

Fig. IX.—Section through Heteromorphic Head, 20 days (text-fig. IV, 8). *e.*, eye; *g.*, gut; *ga.*, ganglion: *op.n.*, optic nerve; *pa.c.*, parenchyma cells.

IV. Discussion.

As was mentioned earlier in the paper, Child (1) has already shown that the presence of at least half the cerebral ganglia is necessary for complete regeneration in *Leptoplana.* The removal of more than half the ganglia causes the production of defective heads. When ganglia and nerve roots are completely removed head-formation is entirely inhibited. The behaviour of *Planaria dorotocephala* was found by Child (2) to be in direct contrast to this. *Planaria dorotocephala* under normal conditions regenerates the anterior end completely from any point of section. It is only when regeneration is suppressed by the addition of anæsthetics or potassium cyanide that defective heads are produced essentially similar to those obtained in *Leptoplana* and *Gunda ulvæ, i.e.,* heads with three to four eyes, or with a single median eye and with the auricles approximated or even fused. With increasing defect of the external appearance Child has shown that there is increasing defect in the regeneration of the ganglia, *e.g.*, in teratomorphic (Child) heads, with single median eye and fused auricles the cephalic ganglia are partially or completely fused; in pieces in which there is no head-regeneration there is similarly no regeneration of the cephalic ganglia. In fact so closely does the parallel run that it seems almost justifiable to assume that in *Leptoplana, Planaria,* and *Gunda* head-regeneration is dependent on the presence of the central nervous system, and that the difference between them is found in the greater power of regeneration possessed by the central nervous system of *Planaria. Planaria* has another characteristic in which it contrasts strongly with the two other genera, and that is in its power of asexual reproduction. It seems quite likely that asexual reproduction and the high capacity for head-formation are both determined by the power of growth and regeneration of the central nervous system.

In *G. ulvæ* the condition is found that the nerve cords can exhibit restoration by backward and forward growth after a cut made at any part of their length, quite independently of the ganglia. The ganglia themselves appear incapable of restoring lost parts, unless one complete ganglion is present, and the restoration of complete heads only occurs if the ganglia are restored.

Considering the inability of animals with badly damaged central nervous system to regenerate heads, the production of heteromorphic heads on short anterior pieces is of great interest. These pieces contain only about one-third of the cerebral ganglia, yet the heads which they regenerate at the posterior end are complete with trifid gut, eyes and auricular processes. It will be remembered that large posterior pieces with defective cerebral ganglia failed to regenerate heads on the oral pole.

In *Planaria maculata* Morgan has obtained heteromorphic heads at any point of section, provided that the fragments which he took were sufficiently short.

From these considerations it seems possible that the mechanism for the restoration of the tail belongs to the body as a whole, while that for restoring the head is an entirely independent one, which may or may not be localised in some part of the body, notably the anterior end. Probably head-formation is dependent on the presence of some specific substance which in *Leptoplana* and *Gunda* is localised in the anterior end, and in *Planaria* is found throughout the length of the body. It would be of great interest and possibly of great importance if a comparison of the relative number of ganglia found in the nerve trunks could be made in these three genera, as it might produce some evidence for or against the suggestion that the head-forming substances may be localised in the central nervous system. At present Morgan's (6) statement that pieces of *Planaria maculata* entirely devoid of nervous system are capable of complete regulation rather bears against the idea that the nervous system is of such great importance in regeneration. The facts presented in this paper do not justify a full discussion of Child's theory of the axial gradient; all that need be said is that to make this work coincide with his hypothesis the axial gradient of *G. ulvæ* must be assumed to slope very steeply at the anterior end. Probably the idea that there is a sudden change in rate of chemical actions in *G. ulvæ* as one passes backwards from the anterior end is not antithetic to the idea of the localisation of some specific enzyme in the front of the body.

Finally it may be noted that this work has disproved the suggestion made by Hallez (4) that Triclads and Polyclads each have their own method of regeneration, for *G. ulvæ,* which is a marine Triclad, behaves like the Polyclads in its method of the restoration of the head.

Summary.

1. Regeneration of posterior parts is independent of the presence of the cerebral ganglia.
2. Lateral regeneration behind the level of the ganglia is independent of their presence. In front of the level of the ganglia at least one complete ganglion must be present for regulation to occur.
3. Anterior regeneration only occurs if the piece contains about two-thirds of both ganglia.
4. Heteromorphic heads are formed by short head-pieces where the cut has passed through the ganglia.
5. *G. ulvæ* differs from most other Triclads and corresponds to Polyclads in its mode of regeneration.

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Investigations dealing with the Phenomena of "Clot" Formations. Part II.—*The Formation of a Gel from Cholate Solutions having Many Properties analogous to those of Cell Membranes.*

By S. B. Schryver.

(Communicated by Prof. V. H. Blackman, F.R.S. Received January 22,—Read February 19, 1914.)

In the first communication under the above title,* attention was called to the fact that solutions of sodium cholate, on warming in the presence of calcium salts, set to a gel, which is not reversible on cooling. It has since been found that calcium salts can be replaced by other salts, such as sodium chloride, magnesium chloride, ammonium sulphate and potassium fluoride, and the clot formation is not therefore due to double decomposition between calcium salts and sodium cholate. The concentrations of sodium, potassium, and magnesium salts necessary to produce the "clot" are, however, much higher than that of calcium chloride, which even in the concentration of N/40 can cause 1-per-cent. sodium cholate to set to a solid gel in about a quarter of an hour at 50°. Sodium and magnesium chlorides produce clot formation at 50°, when their concentration is of about the order of half saturation. It is proposed to investigate the relative clotting power of salt in greater detail later; the present communication deals mainly with the question of the influence of one particular factor on gel formation, viz., on the surface tension of the solution.*

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* 'Roy. Soc. Proc.,' B, vol. 86, p. 460 (1913).

A preliminary account of the clot production by various calcium salts has been given in the first paper (*loc. cit.*). It was then shown that in the case of calcium salts which increase the surface tension of water, increase in the concentration of the salt caused a diminution of the clotting time. In the case of the calcium salts which lowered the surface tension, however, the accelerating effect of the increase in the concentration of the salt was counterbalanced by the diminished surface tension of the solution. In two cases (those of calcium dichloracetate and of the sulphocyanide) an optimal point was found. Increase in the concentration of the calcium salt above and below this point caused a lengthening of the time required for gel formation. In the case of calcium trichloracetate, the length of clotting time progressively increased with increasing concentration within the limits investigated.†

Method of Experiment.—The following was the method of experiment adopted:—1 c.c. of a 4-per-cent. sodium cholate solution and 3 c.c. of the salt solution in the required concentration were heated in separate quartz tubes of 10 c.c. capacity in a thermostat at 50°. As soon as the liquids had attained the temperature of the thermostat, they were rapidly mixed; the salt solution was poured into the cholate solution, and the mixture was then poured back into the tube originally containing the salt alone. This was then clamped in the (transparent) thermostat and watched. The formation of oily globules was the first sign of clot formation. These at first moved rapidly in the liquid, but as they increased in size, motion became less rapid, until, finally, movement was hardly visible. At this point, at short intervals, steel balls of 3/32 inch diameter, such as are used for ball-bearings, were dropped into the tube at short intervals. The time of complete gel formation was taken as that at which a ball stopped dead before it had fallen half-way through the tube. The time was taken by a stop-watch, which was started at the moment of mixing the solutions. Even when the clotting time was 5 minutes, control determinations seldom differed by more than 10 seconds—they usually agreed with one another within 5 seconds. In the first series of experiments on calcium salts, the cholate solution was made by saturating N/100 sodium hydroxide with cholalic acid‡ until a solution neutral to neutral red was obtained. An approximately 4-per-cent. solution was obtained. It was found, however, that cholalic acid is soluble in sodium cholate solution, the amount dissolving varying with the temperature. As this free acid inhibits the clotting, it was found that the clotting time of a solution varied from day to day. For all subsequent experiments, sodium cholate was prepared and a 4-per-cent. solution was made directly from this. The cholate was made by dissolving cholalic acid in 20 times its weight of alcohol, neutralising this solution with sodium ethoxide, heating for a short time on a water-bath and filtering off the first separation of solid, and then evaporating the filtrate. Sodium cholate rapidly separated after a short time, and was filtered off, washed with acetone, and then dried, first on a water-bath, and then over sulphuric acid in a desiccator. It gave a solution in water, acid to phenolphthalein, but slightly alkaline to neutral red.

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* Throughout this communication by "surface tension" is meant surface tension measured against air.

† The surface tension of the salt solutions is affected mainly by the anions. The series of anions employed was that used in the investigations on aggregation ('Roy. Soc. Proc.,' B, vol. 83, p. 96 (1910)).

‡ Prepared pure by the author's method ('Journn. Physiol.,' vol. 44, p. 265 (1912)).

Table I.—Clotting Time of 1-per-cent. Sodium Cholate Solution (in seconds) in presence of Varying Concentrations of different Calcium Salts.

	3N/4.	N/2.	3N/8.	N/4.	N/8.
Chloride	3·0	14	14·0	21	34
Bromide	10·5	14	15·5	21	47
Nitrate	16·0	17	37·0	40	62
Iodide	18·0	17	20·0	24	41
Sulphocyanide	87·5	54	47·0	47	70
Formate	11·0	14	14·0	19	38
Acetate	8·0	10	12·0	19	34
Monochloracetate	24·0	26	33·0	36	77
Dichloracetate	97·0	47	33·0	30	52
Trichloracetate	∞	Incomplete clot in 25 minutes	710·0	170	122

INFLUENCE OF ORGANIC COMPOUNDS ON THE GEL FORMATION.

Many attempts have been made within recent years to correlate physical properties of organic substances with their different biological actions, such as the production of exosmosis, hæmolysis, narcosis, etc.; more especially, their effect on the surface tension of water has been stated to be intimately associated with the production of changes in the cell. Czapek* (in his monograph, January, 1911) claims to have determined the surface tension of the membrane of certain plant cells by showing that exosmosis of tannin takes place, whenever they are immersed in solutions the surface tension of which falls below 0·681 (water = 1). The smaller the amount of a given substance necessary to reduce the surface tension to this figure, the lower is the concentration of that substance which will produce exosmosis. The conceptions of Czapek have been subjected to severe criticism, principally by Vernon, who has shown that there are many exceptions to Czapek's rule. Several substances, especially the narcotics and acetonitrile, produce exosmosis in solutions, the surface tensions of which are appreciably greater than Czapek's critical point. Czapek accounts for the exceptions to his rule by ascribing to them some specific toxic property.

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* See also the numerous papers by Traube.

Vernon has shown that a marked parallelism exists between hæmolytic and narcotic actions and the inhibitory action on indophenol oxydases of animal tissues. Batelli and Mlle. Stern support Vernon's views in their investigations on the so-called oxydones and correlate the various toxic properties with the property of precipitating nucleoproteins.*

In view of the above-mentioned facts and the preliminary experience obtained from the study of the calcium salts as to the action of surface tensions, it was a matter of considerable interest to study the inhibitory action of various organic substances on the formation of the cholate gel, which is derived from chemically pure crystalline products, and which, in thin films, might be regarded as a structure analogous to the cell membranes. As a result it was found that whilst generally those substances which have the greatest power in lowering the surface tension of water have a greater inhibitory action on the gel formation, this rule is by no means absolute, and the chief exceptions are the narcotics and acetonitrile, *i.e.* the very substances which deviate from Czapek's generalisation. There is, moreover, a very close parallelism between the inhibitory action on gel formation and narcotic and other biological reactions. In the following experiments 0·5 c.c. of calcium chloride was diluted to 3 c.c. with water containing a given amount of the substance under investigation, and this was then mixed with 1 c.c. of 4-per-cent. sodium cholate, and the clotting time was determined by the method given above. The concentration of cholate in the mixture was, therefore, 1 per cent., and of the calcium chloride, N/8. By determining the weights as well as the volumes of the organic substances, and also the specific gravity of the solutions, the concentrations in gramme molecules per litre could be calculated.

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* 'Biochem. Zeitsch.,' vol. 51, p. 1 (1913).

Table II.—Clotting Time without addition of Organic Substance, 17 secs.

Per cent. (vol.).	Grm. mol. per litre.	Clotting time, secs.	Per cent. (vol.).	Grm. mol. per litre.	Clotting time, secs.	Per cent. (vol.).	Grm. mol. per litre.	Clotting time, secs.
Ethyl Alcohol.			Propyl Alcohol.			Isopropyl Alcohol.		
2·5	0·437	20	1·25	0·171	21	2·5	0·334	24
3·75	0·656	31	1·875	0·256	38	3·75	0·561	99
5·0	0·875	47	2·5	0·342	72	5·0	0·668	272
6·25	1·093	85	3·125	0·432	198	6·25	0·835	675
7·5	1·312	218	3·75	0·513	405	7·5	1·002	1710
8·75	1·532	514	4·375	0·600	794			
10·0	1·749	805*	5·0	0·684	1200			
11·25	1·968	1190*	5·625	0·770	†			
Butyl Alcohol (Normal).			Secondary Butyl Alcohol.			Tertiary Butyl Alcohol.		
1·25	0·137	38	1·25	0·139	27	1·25	0·136	18
1·5625	0·171	76	2·5	0·277	195	3·125	0·339	60
1·875	0·205	185	3·125	0·346	645	3·75	0·407	105
2·1875	0·239	333	3·75	0·416	Inc.‡	4·375	0·468	270
2·5	0·273	442				5·0	0·543	375
2·8125	0·308	665				5·625	0·611	510
3·125	0·342	Inc.				6·25	0·679	790
						7·5	0·814	Inc.
Amyl Alcohol.			Secondary Amyl Alcohol.			Tertiary Amyl Alcohol.		
0·9375	0·096	71	1·25	0·113	56	1·25	0·115	28
1·25	0·129	210	1·875	0·169	267	1·875	0·173	78
1·5625	0·161	480	2·1875	0·197	810	2·1875	0·202	136
			2·5	0·225	Inc.	2·5	0·230	237
						2·8125	0·259	352
						3·125	0·288	808
						3·25	0·346	Inc.
Allyl Alcohol.			Acetonitrile.			Methyl Ethyl Ketone.		
1·25	0·188	21	2·5	0·495	47	2·5	0·288	59
2·5	0·376	38	3·75	0·703	114	3·125	0·360	107
3·125	0·470	45	4·375	0·867	144	3·75	0·432	173
3·75	0·564	135	5·0	0·991	333	4·375	0·505	362
5·0	0·752	382	5·625	1·115	607	5·0	0·577	695
6·25	0·940	810	6·125	1·239	780	5·625	0·650	910
7·5	1·128	Inc.	7·5	1·406	§	6·25	0·721	1257
						7·5	0·864	Inc.
Methyl Propyl Ketone.			Chloral Hydrate.			Methyl Carbaminate.		
			Per cent. (weight).			Per cent. (weight).		
1·25	0·121	38	0·625	0·038	29	2·5	0·333	33
1·875	0·182	46	1·25	0·076	109	3·75	0·500	50
2·5	0·242	260	1·5625	0·094	210	5·0	0·666	79
2·8125	0·272	496	1·875	0·113	915	6·25	0·833	255
3·125	0·302	607*	2·1875	0·132	‖	7·5	1·000	645
						8·75	1·166	>1800

* With separation of crystals.
† Not complete in half hour.
‡ Inc. indicates incipient clotting in half an hour.
§ Inc. with separation of crystals.
‖ Not quite complete in half hour.

Table II—*continued.*

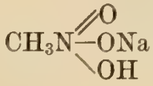
Per cent. (vol.).	Grm. mol. per litre.	Clotting time, secs.	Per cent. (vol.).	Grm. mol. per litre.	Clotting time, secs.	Per cent. (vol.).	Grm. mol. per litre.	Clotting time, secs.
Ethyl Carbaminate.			Propyl Carbaminate.			Witte's Peptone.		
Per cent. (weight).								
1·25	0·140	26	1·25	0·121	36	1·875		213
1·875	0·210	38	1·875	0·182	93	2·5		360
2·5	0·281	56	2·1875	0·212	248	3·125		570
3·75	0·421	147	2·5	0·242	360	3·75		1530
4·875	0·491	296	3·125	0·303	910			
5·0	0·561	564				Phenol.		
5·625	0·632	750						
6·25	0·702	1448				0·625		165
						0·9375		550

Chloroform.

A solution of water saturated with chloroform at 17° contains 0·710 per cent. When 2·5 c.c. of this solution was mixed with 0·5 c.c. N. calcium chloride and 1 c.c. 4-per-cent. cholate solution, the clotting time was not appreciably longer than when no chloroform was present. By diminishing the concentration of the calcium salt to one half, the clotting time was 230 seconds in the presence of saturated chloroform water (2·75 c.c. in 4 = 0·041 grm. mol. per litre), as compared with 45 seconds, the clotting time in the absence of chloroform. The clotting time in presence of 0·064 grm. mol. per litre amyl alcohol, and the same amount of calcium salt, was 155 seconds, and of 0·038 grm. mol. per litre chloral hydrate 194 seconds. The inhibitory action of chloroform is therefore greater than that of amyl alcohol.

Nitromethane.

The action of nitromethane is anomalous. In the presence of 3·75 per cent. the clotting time is 23 seconds, in the presence of 5 per cent. it is 57 seconds, and of 6·25 per cent. it is 42 seconds. It appears to behave more or less like an acid, for in the presence of hydrochloric acid in N/800 concentration, the clotting time of cholate solutions is 41 seconds, in N/400 it is 104 seconds, 3N/800 it is 185 seconds, and in N/200 it is 74 seconds. At the highest of these concentrations the acid is sufficient to cause precipitation of free cholalic acid; on keeping at 50° the precipitate disappears and a gel then forms. The lower concentrations produce no separation of free organic acid in form visible to the naked eye. The nitromethane possibly forms the salt



$\mathrm{CH_3N}\overset{\mathrm{O}}{\underset{\mathrm{OH}}{-\mathrm{ONa}}}$ by double decomposition.

Polyhydroxy-Derivatives.

The inhibitory action of these substances is small, as is shown by the following examples:—

Substance.	Per cent. (weight).	Clotting time, secs.
Ethylene glycol	12·5	281
Propylene „	8·75	385 (with separation of crystals)
Glycerol	12·5	148
Sucrose	12·5	29
Dextrose	12·5	30
Dextrin	12·5	258
„	10·0	100

Discussion of Results.

Whilst it cannot be denied that those substances which lower most markedly the surface tension of water have, as a rule, the greatest tendency to exert an inhibitory effect on the formation of the cholate gel, the law is not by any means an absolute one. The exceptions are precisely the ones which deviate from Czapek's generalisation. Acetonitrile, which lowers the surface tension of water but little, has a greater inhibitory power than ethyl alcohol, which lowers it much more. The deviation from the rule is shown in a very marked manner also by the typical narcotics, chloral, chloroform, and (in the experiment on gel formation) by urethane. There is, in fact, a striking parallelism between inhibition of gel formation, narcotic and hæmolytic actions and production of tannin exosmosis, which is well exhibited in the following table. The various substances are arranged

Substances in decreasing order of gel-inhibiting action.	Critical narcotic concentration. Grm. mol. per litre.
CHLOROFORM	0·0012
CHLORALHYDRATE	0·02
Isoamyl alcohol	0·023
Secondary amyl alcohol (methyl propyl carbinol)	—
Tertiary amyl alcohol (dimethyl ethyl carbinol)	0·037
Propyl carbaminate	—
Normal butyl alcohol	0·038
(Methyl propyl ketone	0·019)
Isobutyl alcohol	0·045
Normal propyl alcohol	0·11
URETHANE	0·041
Tertiary butyl alcohol	0·13
Isopropyl alcohol	0·13
Allyl alcohol	0·13
Methyl carbaminate	0·27
ACETONITRILE	0·36
Ethyl alcohol	0·3

in the order in which they inhibit the gel formation, the more active substances being placed first in the list. The numbers given are the strengths in which they produce narcosis of tadpoles according to Overton.

The concordance between the gel inhibitory action and the narcotic action is striking. Methyl propyl ketone is an apparent exception, but gel-inhibiting action of this substance cannot be accurately determined, as in relatively small concentrations it causes the formation of crystals. The same is true for ethyl alcohol in higher concentrations. Normal propyl alcohol should follow instead of preceding urethane. The substances showing a marked deviation from the surface tension generalisation are indicated in large type.*

General Summary and Conclusions.

The inhibition of gel formation may be assumed to be due to adsorption of various substances from solution, which prevent the formation of larger aggregates, which constitute the gel.† The adsorbability of those substances cannot be determined by their effect alone in lowering the surface tension of water. Czapek has assumed that certain plant cells have a lipoid membrane, with a surface tension of about 0·681 (water = 1), and that, when they are immersed in an aqueous solution, the surface tension of which has been reduced to below this figure, exosmosis of complex molecules takes place, owing to the changes in the lipoid membrane. Czapek found, however, that certain substances deviated from his rule. To these he ascribed a specific toxic action on the cell. In view of the fact that these same substances show a deviation also from a surface tension rule in their inhibitory action on the formation of the cholate gel, a phenomenon from which specific biological action is excluded, the purely mechanical conception of cytolysis, as propounded by Czapek, is clearly no longer tenable. Nor do the results in the above paper support the Overton-Meyer lipoid hypothesis. Although the lipoid soluble substances have, as a rule, the greatest inhibitory action on gel formation, the gel itself cannot, by any extension of the meaning of the term, be described as a lipoid. It is formed from the salt of an acid, which is generally insoluble in organic solvents, in which even the free acid itself is only slightly soluble. The results suggest that the semipermeability of the cell may owe its properties to the presence of some gel-forming substance which has not yet been isolated, and which need be neither lipoid nor protein. Such a gel need not, furthermore, be continuous, but may simply form a matrix, holding together proteins and lipoids and other cell constituents. The protoplasm itself may exert its normal functions only when its constituents are held in such a matrix. The amount of substance to which the gel formation may be due need be present only in very small quantities. A solid gel has been obtained with ½-per-cent. solutions of sodium cholate, but the author, in conjunction with Dr. E. Graf von Schönborn (in a preliminary communication to the Biochemical Society last May), has shown that solid gels are formed from sodium deoxycholeate (another bile acid), when the concentration does not exceed 1 in 1000.

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* Several estimations of the surface tensions of solutions have been made by different observers. Czapek's own numbers have been adopted. In arranging the above table the approximate dilutions which delay gel formation 15 minutes have been ascertained. The surface tensions of these dilutions in water lies normally between 0·5 and 0·67 (water = 1). The substances indicated in capitals deviated markedly from these numbers.

† Compare Schryver, 'Roy. Soc. Proc.,' B, vol. 83, p. 96 (1910).

Various other problems arise from the study of these gels. Attention has been called to the fact that relatively large quantities of sodium and magnesium salts are necessary to produce gel formation as compared with those of calcium salts. These facts offer a suggestion as to the antagonism of calcium salts to the toxic action of sodium and magnesium salts, as has been observed by Loeb, in the case of fundulus eggs, and of which many other biological examples exist. The replacement of a calcium salt by sodium or magnesium salts may render a gel unstable. It is proposed to investigate phenomena of this description. In all the above experiments a large excess of calcium salts has been employed in gel formation, in order to accelerate this phenomenon. To obtain results more analogous to the various biological phenomena, it will be necessary to study the action of various reagents on the gel when in thin membranes, and under conditions under which excess of calcium salts can be readily removed.* Preliminary experiments indicate that under such conditions the gel may be reversed. Work is proceeding in this direction, and it is also proposed to employ the gels for the study of various phenomena of permeability.

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* In the above-described experiments the inhibitory action of various substances on a membrane (or gel) formation has been studied. It has been assumed in these arguments that the more powerful this particular action of a given substance is, the greater will be its disaggregating action on an already formed membrane (or gel).

A New Malaria Parasite of Man.

By J. W. W. Stephens, M.D., Sir Alfred Jones Professor of Tropical Medicine, University of Liverpool.

(Communicated by Sir R. Ross, K.C.B., F.R.S. Received January 19,—Read February 19, 1914.)

[Plates 14–16.]

In the autumn of 1913 Major Kenrick, I.M.S., kindly sent me, from Pachmari, Central Provinces, India, a blood slide from a native child, containing numerous malaria parasites. On examining these, which I at first took to be malignant tertian parasites, the suspicion arose in my mind that there was something peculiar about their appearance. I happened just previously to have been studying a blood slide from Rhodesia, containing very numerous malignant tertian parasites. The peculiarity of the Indian parasite, as far as I could at first define it, was that it was an irregular parasite as compared with the regular, almost monotonous, contour of the "rings" of the malignant tertian parasite (*Plasmodium falciparum*). I proceeded then to study the Indian parasite more carefully; and, after daily observations for many weeks of it, and of control malignant tertian parasites from various sources, I came definitely to the conclusion that it was unlike any malignant tertian parasite that I had ever seen or that I could find figured in the text-books or journals. I also considered carefully the possibility of its being the simple tertian parasite, but to this point I shall return later. During this study, in order to fix my impressions, I drew 150 consecutive parasites from the Indian slide and the Rhodesian slide respectively, as the former appeared in the field of view of an ocular so restricted by placing a diaphragm in it that only half a dozen red cells were visible in the field at a time, thus effectively preventing any selection on my part. I reproduce as pen-and-ink drawings 35 of each series taken at random, as they show very well in a general way the different aspect of the two parasites. For the same purpose I also drew a number of young simple tertian parasites.

I now proceed to define as far as possible in detail the peculiarities of this parasite.

1. It is *extremely amœboid* (judging from the stained specimens). Thin processes often extend across the cell or occur as long tails to more or less ring-shaped bodies. These processes may be several in number, and may

give the parasite most peculiar fantastic shapes like that of an irregular web or mesh.

2. The *cytoplasm* is always scanty, *i.e.* the individual amœboid processes are delicate or thin, and the parasite has but little bulk, or density. While forms resembling "rings" do occur, yet, owing to the abundance of all kinds of irregular forms, it is certainly difficult to find quite typical "signet" rings. Laterally applied parasites (*accolés* of French authors) also occur, but in them the chromatin is not dot-like, as it usually is in the malignant tertian, but practically always rod-like.

3. The *nuclear chromatin* is out of proportion to the volume of the parasite. It takes the form of bars or rods, strands, curves, forks, patches, etc.; the occurrence of the chromatin in a dot, as in the "ring" forms of other species, is rare. In the web-like protoplasmic processes mentioned above there may be seen several chromatin strands, and not uncommonly one observes a minute dot of chromatin some way from the parasite, or between two portions of the parasite, though the protoplasmic process connecting it with the main mass or masses is so thin as to be invisible. The chromatin masses are frequently angular, the angles jutting into the points at which an amœboid process is given off. Abundance of, and marked irregularity in distribution of, the chromatin masses are characteristic of this parasite.

I reproduce in a coloured plate the peculiar forms of this parasite, as it is very difficult, if not impossible, to describe them in words.

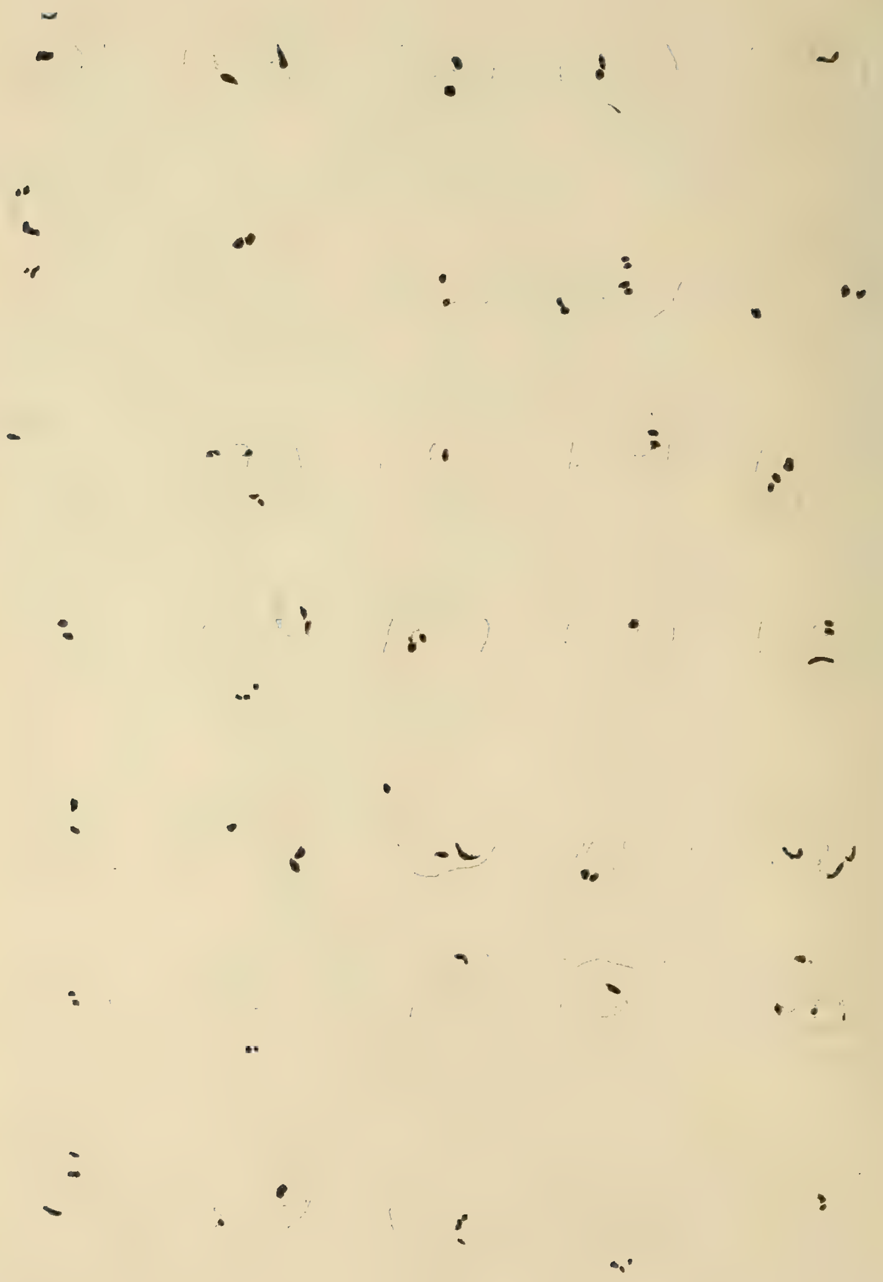
I next consider in what respects this parasite in my opinion differs from the hitherto described parasites of malaria.

Malignant Tertian Parasite.—It differs from this

(1) In its amœboid activity. In the case of the malignant tertian parasite a certain amount of amœboid activity is observable, giving rise to "star-fish" shapes, and to somewhat irregular or even bacillary forms; but the activity is not comparable with that of this parasite, which has for this reason a most strange and peculiar appearance. The picture produced by the splash of a drop of ink on paper may suggest some of the forms seen.

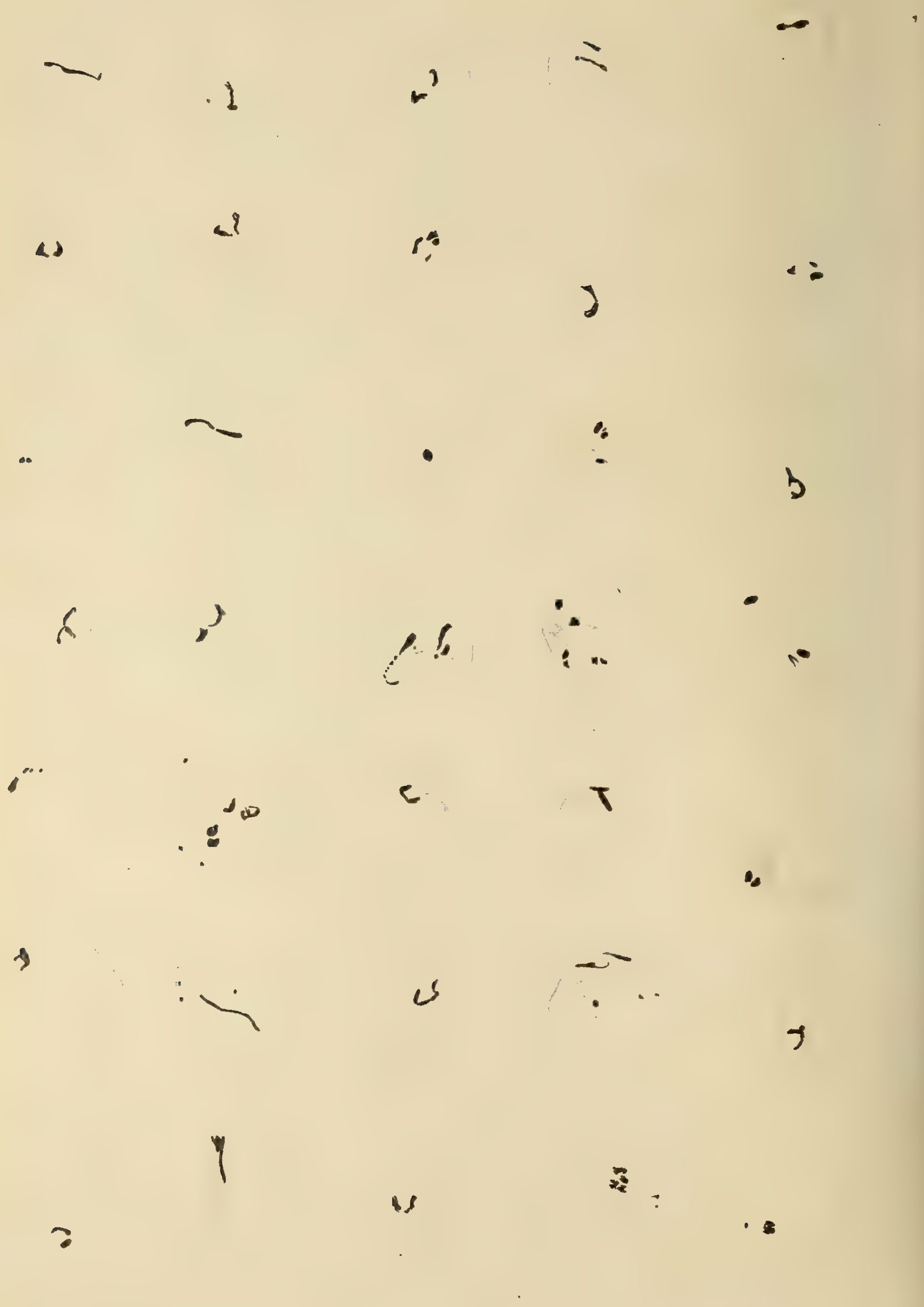
(2) In the abundance and irregularity of nuclear matter. This, as the coloured plate shows, is very different from what one finds in the malignant tertian parasite, where the term "signet rings" well expresses the general morphology. The quotidian parasite, if such exists, differs so slightly morphologically from the malignant tertian parasite that the differences just pointed out between this Indian parasite and the malignant tertian apply equally to it.

Simple Tertian Parasite.—It differs from this in the following respects:—



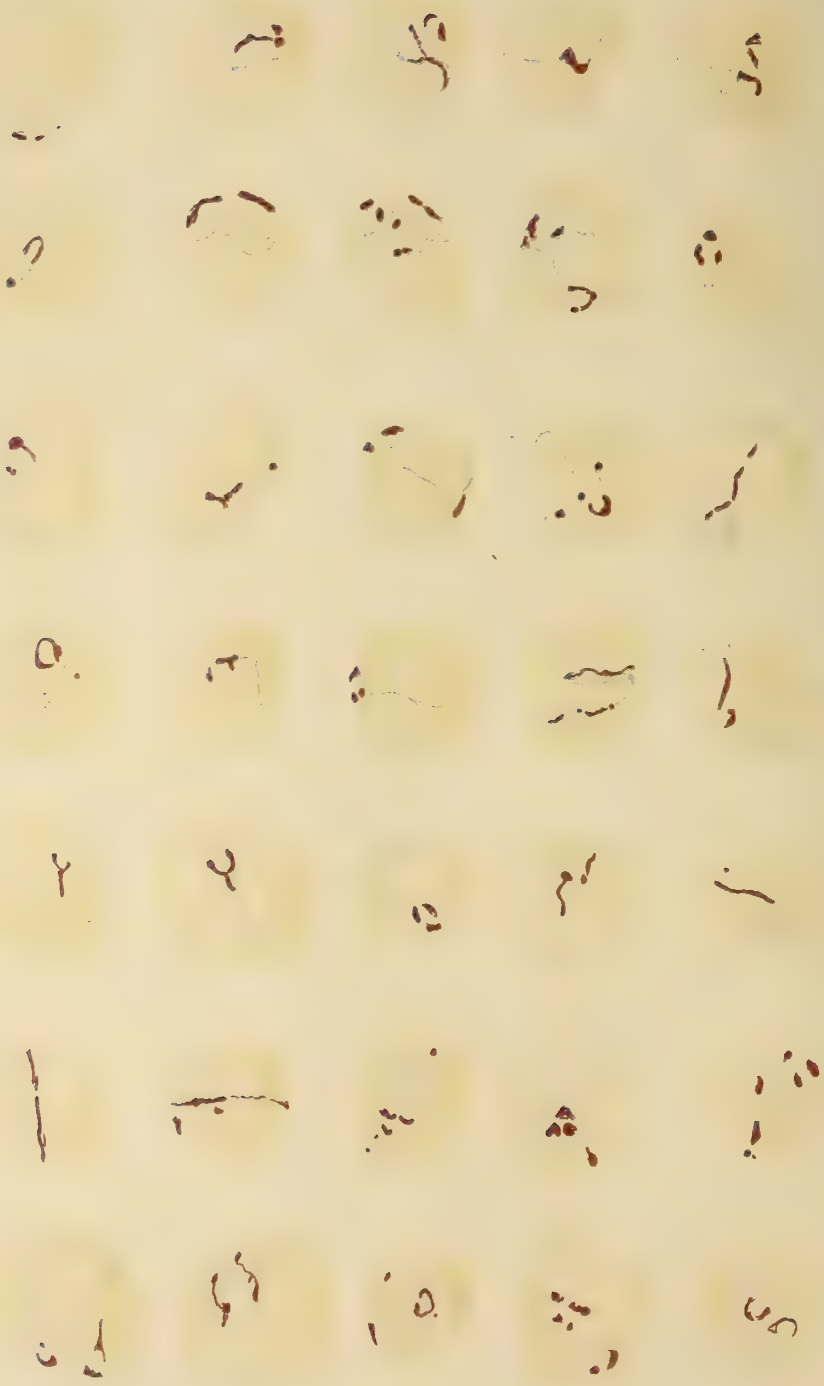
Plasmodium falciparum.

x 2300. (approx.)



Plasmodium tenue.

x 2300. (approx.)



Plasmodium tenue. X 2300. (approx.)

(1) Its *bulk* is much less, *i.e.* it is a smaller parasite.

(2) The *amœboid processes* are far more delicate.

(3) The *chromatin* shows a relative abundance, an irregularity and a peculiarity of arrangement (*e.g.* strands, rods and bars) not seen in the simple tertian parasite.

(4) *Typical rings* are absent or exceedingly rare.

I am not sure whether this parasite enlarges the cell, as, although in some cases I found infected cells larger than non-infected ones in their vicinity, in other cases the reverse held good. I am uncertain also whether it is pigmented or not. I have found no parasites in which I could certainly detect pigment; but, on the other hand, I found three pigmented leucocytes in the film, which leucocytes may be associated with this parasite or may result from an associated infection. Finally, I am in doubt as to whether it produces any change in the red cell such as Schüffner's dots. During the course of my examination of this film I must have observed many thousand parasites, but among these I encountered only one infected cell which was clearly enlarged and which showed Schüffner's dots. The bulk of this parasite was much greater than that of any other I had seen, whereas the chromatin masses (two in number, one large, one small) were small compared to the bulk of the parasite. Although I could detect no pigment in this parasite I was not otherwise able to distinguish it from a simple tertian parasite.* These points then must remain unsettled until further material is forthcoming.

Quartan.—Its amœboid activity and its tenuity easily distinguish it from this species.

After a prolonged study of this parasite I believe then that its morphology differentiates it from any malaria parasite of man yet described.

I propose to call it *Plasmodium tenue.*

DESCRIPTION OF PLATES.

The figures were all drawn with an Abbé camera lucida at the same magnification, ×2300 (approx.).

Plate 14.—*Plasmodium falciparum.* Blood slide from Rhodesia; 35 parasites drawn at random.

Plate 15.—*Plasmodium tenue.* Blood slide from Central Provinces, India; 35 parasites drawn at random.

Plate 16.—*Plasmodium tenue.* Illustrating the irregularity of form of this parasite.

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* I incline to the view, however, that this large form belongs to the other irregular forms, and hence that this parasite enlarges the cell and produces a stippling in it, and hence also that it has affinities with the simple tertian parasite, and *Plasmodium canis* of the dog rather than with the malignant tertian parasite.

Formaldehyde as an Oxidation Product of Chlorophyll Extracts.

By Charles Horne Warner, B.Sc., F.I.C.

(Communicated by Prof. V. H. Blackman, F.R.S. Received February 3,—Read March 5, 1914.)

(From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology.)

Of recent years the action on carbon dioxide of chlorophyll *in vitro* has assumed some importance as possibly throwing light on the nature of the photo-synthetic process of green plants. Thus Usher and Priestley* have stated that films of extracted chlorophyll in the presence of moist air and carbon dioxide produce formaldehyde and hydrogen peroxide under the influence of light. The earlier work of these authors has been adversely commented upon by several writers, notably by Ewart,† to whose criticisms Usher and Priestley have replied with a number of additional experiments and arguments, referring also to the work of Schryver,‡ subsequent to that of Ewart, as affording strong confirmation of their views as far as the synthesis of the aldehyde is concerned. The facts set forth in the present paper came to light during an attempt to confirm and extend the observations of Usher and Priestley and of Schryver.

Grass was extracted with alcohol, usually in the cold and in the presence of calcium carbonate. In some experiments the alcoholic liquid was evaporated to dryness under reduced pressure and the residue extracted with ether; in others a solution of chlorophyll in light petroleum was obtained by shaking the alcoholic solution with that liquid. The method of experiment was based upon that described by Schryver, the ether or petroleum extract being allowed to evaporate on glass plates and exposed to light under the various conditions to be mentioned below. As was the case in the later experiments of Usher and Priestley themselves, the test which has been relied upon for the detection of formaldehyde is the very delicate one devised by Schryver, who has found that the reaction is not given by such other members of the series as have been examined up to the present. It has been assumed in the course of these experiments, as in the work of the investigators already mentioned, that the aldehyde produced is formaldehyde, but it is very important that the possibility of the observed effects being due to some other aldehyde or to a mixture of aldehydes should be borne in mind. This point still requires investigation.

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* 'Roy. Soc. Proc.,' B, vol. 77, p. 369 (1906); vol. 78, p. 318 (1906); and vol. 84, p. 101 (1911).

† *Ibid.*, vol. 80, p. 30 (1908).

‡ *Ibid.*, vol. 82, p. 226 (1910).

The Production of Formaldehyde by Chlorophyll Extracts in Air.

In 14 experiments films of chlorophyll extract together with tubes of soda water were exposed to light in glass jars with well-fitting stoppers greased with vaseline. The effect both of electric light (eight experiments) and sunlight (six experiments) was investigated. In the former case the source consisted usually of one or two 32 c.p. filament lamps which were separated from the vessels containing the films by a glass tank, 2 inches thick, through which cold water flowed; the exposures varied from 6 to 70 hours.* The films illuminated by sunlight were exposed outside a south window for periods ranging from two and a quarter hours of bright sunshine to seven days bright at intervals (March 4–11, 1913).

Similar films were always exposed in jars containing tubes of potassium hydroxide solution. These control films were allowed to stand in the dark *in vacuo* over lime for several days before being rapidly placed in the jars, and then for several more days over the potash solution before exposure to light. In all the films a development of formaldehyde was observed, the solutions becoming very decidedly coloured in most instances when the test was applied. In 10 of these experiments no difference could be detected between the amounts of aldehyde formed in the presence of carbon dioxide and in its absence. In three there was a very slight excess in the films exposed to carbon dioxide as compared with the control films, but the difference was so small as to be barely perceptible, while in the remaining experiment slightly more aldehyde was found in the film which had not been exposed to carbon dioxide.

Subsequently three experiments were carried out with somewhat greater precautions, sealed glass tubes replacing the stoppered jars throughout. The exposures varied from one to two and a half hours of bright summer sunshine, and in each case the production of formaldehyde was very evident. In one instance it was impossible to distinguish between the amounts formed in the presence and absence of carbon dioxide, while in the other two there was slightly more formaldehyde produced in the films which had been exposed over potash solution. In all of the 17 experiments control films were also examined which had remained in the dark for equal periods, and in no case was formaldehyde found in such a film whether carbon dioxide had been present or not.

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* During the daytime these films received diffuse sunlight in addition to artificial light.

In some, at least, of the experiments which yielded indications of the photo-synthesis of formaldehyde to previous investigators, either lime, soda-lime, or solid potash was used to obtain a control atmosphere free from carbon dioxide. A number of additional experiments have been carried out under these conditions, which are, however, entirely unsatisfactory, since the effect of a moist atmosphere containing carbon dioxide is compared with that of dry carbon-dioxide-free air. In a few of these experiments, particularly when short exposures to rather dull light were given, distinctly more formaldehyde was formed in the films exposed to carbon dioxide than in the controls; in the remainder no appreciable difference was observed.

In order to further investigate the influence of moisture upon the production of formaldehyde, several experiments were performed in such a way that the effects of moist and dry carbon-dioxide-free air could be compared. Films were exposed in pairs, one in air over potash solution, and the other in air over lime or soda-lime, a tube of concentrated sulphuric acid being present in addition in some cases. In this series also it was noticed in some instances that the comparatively dry films contained distinctly less formaldehyde than the moist ones. These experiments, taken as a whole, appear to show that, while there is no appreciable difference between the amounts produced by such chlorophyll films in moist air containing carbon dioxide and in similar air free from that gas (such differences as have been observed being chiefly due, as shown by later experiments, to the air diluted by carbon dioxide causing less oxidation), the aldehyde is formed more readily in moist than in dry air.

It is evident that formaldehyde was being produced in these experiments as the result of photo-chemical decomposition of the films. This is one of the points to which attention was drawn by Ewart (*loc. cit.*), who found that an aldehyde was formed by chlorophyll under the action of light both in air in which carbon dioxide was present and in that from which it was absent, nor could he observe any difference in colour intensity between the two cases when Schiff's reaction was applied. In this connection Ewart relied chiefly upon observations on killed leaves, a method of experimentation very difficult to control; in addition to chlorophyll, the number of substances present, some of which (such as organic acids) may give formaldehyde on exposure to light, is very large, much greater than in an ether or petroleum extract; also, it is considerably more difficult to ensure freedom from carbon dioxide for control experiments in such leaf material than in the thin films obtained in the manner described. Again, the rosaniline test, apart from its comparative lack of sensitiveness and the fact that it cannot be used quantitatively, must be regarded as most unsatisfactory for work of this type even when applied with great care, since it has no specificity, and the escape of sulphur dioxide from the solution causes the appearance of the red colour in the absence of aldehyde.

Illumination of Films in an Atmosphere of Nitrogen.

It was next necessary to determine the nature of the decomposition described above, in order to eliminate it, and thus ascertain whether photosynthesis was taking place at the same time. With this object in view, films in sealed glass tubes containing alkaline pyrogallate solution were exposed to bright sunlight for periods of one, one and a half, two, and two and a quarter hours, and to sunshine intermittently bright for periods of five and a half and twelve hours (twice). No trace of formaldehyde could be detected in any of these films after exposure. In carbon-dioxide-free films which received equal exposures over potash solution, the amounts formed were always very considerable.

A tube containing recently boiled water and a film of chlorophyll extract was six times alternately exhausted and filled with nitrogen, which had been passed through potash solution and over copper heated to redness. After the final filling the tube was sealed off and exposed to bright sunlight for two hours, and to diffuse sunlight for a further period of three hours. On examining the film, again no trace of formaldehyde could be found, although the quantity which had been produced in a film similarly exposed over a solution of potassium hydroxide was most distinct. Both films were allowed to stand *in vacuo* over soda-lime and pyrogallate solution in the dark for three days before being quickly introduced into the tubes, and the sealed vessels remained for four days more in the dark before exposure.

Illumination of Films in Carbon Dioxide free from Oxygen.

Four tubes containing freshly boiled water and chlorophyll extract films were six times alternately exhausted and filled with carbon dioxide freed from oxygen by means of red hot copper, and sealed off after the sixth filling. The following exposures were given: (*a*) 12 hours of sunlight, bright at intervals; (*b*) 11 hours of bright and 6 hours of diffuse sunlight; (*c*) 25 hours of bright and 6 hours of diffuse sunlight; and (*d*) 17 days of intermittent sunshine (July 16 to August 2, 1913). In no case was there any evidence that formaldehyde had been formed in the exposed films, although in each case control films, carefully freed from carbon dioxide and exposed over potash solution under the same conditions, showed a very marked formation of the aldehyde.

Usher and Priestley* thermo-electrically determined the temperatures of two similar films of chlorophyll extract exposed to light, carbon dioxide being present in the surrounding atmosphere in one case and absent in the other. They found that the temperature of the film in contact with carbon dioxide was the lower, a result which they regard as evidence that synthesis had taken place consequent on the absorption of energy by the film. This result is in no way conclusive, and may be quite valueless, since, apart from the difficulties due to the conditions of the experiment, there is the fact that oxidation must have been proceeding in both cases, and the observed temperature difference was in all probability due to the excess of oxidation of the film in air as compared with that of the one in air diluted with carbon dioxide. The greater proportion of oxygen in the air from which carbon dioxide was absent would certainly account for the observation that "the film in CO_2-free air was scorched and destroyed sooner than the other." The experiment should be repeated with nitrogen as an atmosphere for the control film.

The Bleaching of Chlorophyll.

Chlorophyll in air becomes bleached by light both in the presence and absence of carbon dioxide, and it has been found that formaldehyde has been produced whenever bleaching has occurred. The bleaching was not more marked in moist air containing carbon dioxide than in air standing over a solution of potassium hydroxide, but when the effect of air containing appreciable quantities of water vapour was compared with that of an atmosphere relatively dry, it was found that the degree of decolorisation (especially in the case of rather short exposures to comparatively dull light) was somewhat greater under the moist conditions. In all of the experiments previously mentioned in which films were exposed in tubes containing no oxygen, including the four for which oxygen-free carbon dioxide was used, no bleaching could be detected. It will be observed that these results agree with those which were described when the production of formaldehyde was under consideration.

The Formation of Hydrogen Peroxide.

The observations of Usher and Priestley on the action of sheep's liver catalase in preventing the bleaching of chlorophyll have been confirmed. These observations point to the conclusion that the bleaching is due to the oxidising action of hydrogen peroxide. Less important evidence tending in the same direction has been obtained by treating films which have been exposed to light and air with potassium iodide, ferrous sulphate and acetic acid. A small amount of iodine was slowly liberated, while films which had been kept in the dark when treated with the same reagents caused no liberation in an equal time. A film which had been illuminated in a sealed tube containing moist oxygen-free carbon dioxide was not bleached and did not give the above reaction, although iodine was set free by a film similarly exposed in air over potash solution; this film was very distinctly bleached. It is, of course, not to be expected that such an unstable substance as hydrogen peroxide would accumulate in any quantity in these films.

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* 'Roy. Soc. Proc.,' B, vol. 84, p. 107 (1911).

There thus seems to be good ground for believing that the bleaching of chlorophyll in oxygen is due partially, if not entirely, to the action of hydrogen peroxide. Since bleaching does not occur in oxygen-free carbon dioxide, however, there is no evidence that carbon dioxide plays any part in the formation of the peroxide, which substance is obviously produced through the agency of atmospheric oxygen. The somewhat greater readiness with which bleaching takes place in moist air as compared with air which is relatively dry, is doubtless related to these facts, although it would seem that the change can be effected in the presence of only a very small amount of water.

Usher and Priestley* found that chlorophyll films in a carbon-dioxide-containing atmosphere which previously produced no effect on Beyerinck's "luminous" bacteria, caused glowing after exposure to light. This does not by any means necessarily point, as these authors consider, to the decomposition of carbon dioxide with the formation of oxygen or hydrogen peroxide. It is more probable that, although the gas did not originally contain sufficient oxygen to cause visible luminosity of the bacteria, sufficient was present to form hydrogen peroxide under the action of light, and the peroxide, according to Usher and Priestley, has more effect upon these organisms than oxygen.† The same criticism is applicable to Molisch's‡ statement that assimilation can be carried out by chloroplasts from dried and apparently lifeless cells, and probably to other observations depending upon the use of bacteria also.

Since the production of formaldehyde always accompanies the disappearance of the green colour of chlorophyll in air, it is probable that in this bleaching process hydrogen peroxide oxidises chlorophyll with the formation of formaldehyde among other colourless products, and in support of this view it may be mentioned that the aldehyde is formed in the dark when films of chlorophyll extract are immersed in hydrogen peroxide solution.

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* 'Roy. Soc. Proc.,' B, vol. 84, p. 105 (1911).

† Usher and Priestley found that a certain amount of glowing was produced in the presence of catalase; hence it may be argued that the effect is not due to the action of hydrogen peroxide formed in the manner here suggested. It is practically impossible, however, that under the conditions of the experiment (*q.v.*) the catalase could render the peroxide entirely ineffective.

‡ 'Bot. Zeit.,' vol. 62, p. 1 (1904).

Whether these transformations play any important part in the metabolism of the plant remains to be seen. Possibly some group in the chlorophyll molecule suffers decomposition, thereby liberating formaldehyde, and is then regenerated under the action of carbon dioxide, but the occurrence in the tissues of catalases, which may partially or wholly prevent any such oxidation in the living leaf, must be borne in mind.

The observations of Bach and Chodat,* which appear to have escaped attention in this relation, to the effect that plants (in their experiments, fungi) are able to live in a medium containing 0·68 per cent. of hydrogen peroxide, may be found to have an important bearing upon the subject under discussion.

Experiments testing the possibility of the photo-synthesis of formaldehyde by colloidal chlorophyll in the presence of plant catalases and other enzymes, *i.e.* under conditions more nearly approaching those of assimilating tissues, might yield interesting results.

Films of Carotin Extract.

A few preliminary experiments have been carried out with films of carotin extract. Carrots were extracted with hot alcohol and the liquid was shaken with light petroleum, the petroleum solution being then allowed to evaporate on glass plates. When such films are exposed to air they become bleached both in the light and in the dark,† and formaldehyde is produced in both cases. A carotin film exposed to light in a sealed tube containing moist carbon dioxide free from oxygen was not bleached and showed no evidence of formaldehyde development, while a similar film exposed over potash solution beside the first for the same time became completely bleached and gave a decided reaction for the aldehyde.

Experiments are now being arranged by means of which it is hoped that the formation of hydrogen peroxide by chlorophyll and possibly by carotin and other similar substances, and their derivatives, may be more or less quantitatively investigated.

Thus far the experiments have been confined to crude chlorophyll- and carotin-containing extracts prepared as described; it is intended shortly to investigate similarly the behaviour in this relation of the isolated leaf pigments and some of their derivatives. This is especially important in view of the very recent work of Spoehr,* who has shown that various acids which occur in the leaves of succulent plants may be decomposed by light, yielding formaldehyde. A decomposition such as this would well account for the formaldehyde which Kimpflin† found in the leaves of *Agave mexicana*. Again, Neuberg‡ has shown that a number of substances, under the action of light and in the presence of an optical sensitiser, form this aldehyde; indeed it is probable that there are in the plant many substances which under suitable conditions can give rise to formaldehyde or to hydrogen peroxide.

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* 'Biochem. Centralblat.,' vol. 1, p. 417 (1903).

† Compare Willstätter and Escher, 'Zeit. Phys. Chem.,' vol. 64, p. 47 (1910).

Summary.

1. The photo-chemical development of formaldehyde, which has been observed to occur in films of chlorophyll extract in contact with air containing carbon dioxide and water vapour, is due solely to the decomposition of the films under the action of the oxygen of the air. No formaldehyde is produced when such films are illuminated in a moist atmosphere of nitrogen or of carbon dioxide. There is thus at present no evidence for the photo-synthesis of the aldehyde from carbon dioxide by chlorophyll outside the plant.
2. The above oxidation is accompanied by the bleaching of the films, and appears to be effected by the action of hydrogen peroxide, in the formation of which carbon dioxide can have no share, since there is no decolorisation in moist carbon dioxide free from oxygen.
3. The bleaching (oxidation) of films of carotin extract is also associated with the production of formaldehyde.

Since the experiments are being continued along the lines indicated, a full discussion of the results obtained is for the present deferred. In conclusion, the author wishes to express his indebtedness to Prof. V. H. Blackman, at whose suggestion this work was undertaken, for his very valuable help throughout the course of the research.

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* 'Biochem. Zeitschr.,' vol. 57, p. 95 (1913).

† 'Comptes Rendus,' vol. 150, p. 529 (1910).

‡ 'Biochem. Zeitschr.,' vol. 13, p. 305 (1908).

The Action of Light on Chlorophyll.

By Harold Wager, F.R.S.

(Received February 6,—Read March 5, 1914.)

The chemical changes brought about by light in the green leaf leading to the production of sugars and starch from carbon dioxide and water are still far from being clearly understood.

To what extent the chlorophyll takes part in this process, whether it simply performs the function of bringing the rays of light into contact with the carbon dioxide and water in such a way as to enable them to effect a synthesis of these two compounds, or whether the chlorophyll itself initiates these changes by its own chemical decomposition, are problems still unsolved.

It is a well-known fact that solutions of chlorophyll in the presence of oxygen become decolorised by light, and Pringsheim showed that the chlorophyll in a living leaf becomes rapidly blanched when submitted to the action of an intense light focused through a lens.*

The earliest observations on the destructive effect of light on chlorophyll appear to be those of Sir John Herschel, who in a series of papers† published more than 60 years ago described many interesting experiments on the action of the rays of the solar spectrum on the vegetable colours expressed from the petals and leaves of plants. From these experiments he concludes that (1) the action of light destroys colour, either totally, or leaving a residual tint on which it has no further or much slower action; (2) the action of the spectrum is confined, or nearly so, to the visible rays, as distinguished from the ultra-violet and ultra-red rays, which are ineffective; and (3) the rays effective in destroying a given tint are, in a great many cases, complementary to the tint destroyed. He pointed out that the green colouring matter expressed from leaves and spread on paper shows, as in the elder, a maximum of action, as indicated by the destruction of colour, in the red rays, from which the action falls off rapidly with a slight intermediate maximum in the region of the yellow, then falls off again, and about the termination of the green again increases, reaching another maximum in the blue violet, after which it falls off again, gradually, and ceases to be traceable as the termination of the violet is reached. He points out that "photographic pictures may be taken readily on such papers, half an hour in good sun sufficing; but the glairy nature of the juices prevents their being evenly tinted, and spoils their beauty." He did not experiment with chlorophyll in a state of purity, owing to the nicety required in its preparation.

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* 'Pringsheim's Jahrb.,' 1881 and 1882.

† 'Phil. Trans.,' 1840, 1842; 'Phil. Mag.,' 1843.

There is evidence to show that, under the influence of light, the chlorophyll in a living cell is constantly being destroyed, but that under normal conditions the leaves remain green, the chlorophyll being reconstructed as fast as it is destroyed. Thus when leaves are exposed to a stronger light than usual, they become paler in colour, probably owing to the fact that under these conditions the chlorophyll is destroyed at a more rapid rate than it is reconstructed. This is frequently observed in the leaves of shade plants when exposed to bright sunlight, and is also observed in Algæ such as Spirogyra which accumulate near the surface of water in the intense light of the sun during the summer months.

Thus Ewart[*] states that "when green leaves are exposed to sunlight, the decomposition of the chlorophyll goes on more rapidly than its production, though the amount of chlorophyll decomposed is insufficient to cause a change in the coloration visible to the eye."

Stahl came to the conclusion[†] that the exposure of leaves to direct sunlight for several hours gave no indication of the decomposition of chlorophyll.

Keeble showed, however, that leaves exposed to bright sunlight gave a weaker solution of chlorophyll in alcohol than similar leaves kept in the shade.[‡] Many other experiments support this view, notably those of Ewart[§] conducted on plants both in temperate and in tropical regions. Lubimenko[||] has also shown that the quantity of chlorophyll in a leaf varies with the intensity of the light.

It is usually assumed that the decomposition of chlorophyll is bound up in some way with the photo-synthesis of CO_2 and water, but as it is usually considered to be more or less indirectly one of the results of photo-synthesis, a sort of by-product as it were, very little attention has, so far as I know, been paid to the products of its photo-decomposition.

Timiriazeff pointed out[¶] that chlorophyll is a true optical sensitiser in that it absorbs radiant energy, and is at the same time an absorbent of one or more of the products by which the bleaching is then brought about. The function of chlorophyll is to decompose carbon dioxide; the chlorophyll absorbs the rays of greatest energy and transmits this energy to the molecules of carbon dioxide.

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[*] 'Linn. Journ. Bot.,' vol. 31 (1895–97).

[†] 'Ann. du Jard. Bot. de Buitenzorg,' vol. 11 (1893).

[‡] 'Ann. Bot.,' vol. 9 (1895).

[§] 'Journ. Linn. Soc. Bot.,' vol. 31; 'Ann. Bot.,' vol. 11 (1897); 'Ann. Bot.,' vol. 12 (1898); see also references in Pfeffer's 'Physiology,' Eng. Ed., vol. 1, p. 334.

[||] 'Ann. Sci. Nat. Bot.,' 1908.

[¶] 'Comptes Rendus,' 1885, and 'Ann. Sci. Nat. Bot.,' 1885; see also 'Roy. Soc. Proc.,' 1903.

Again, according to the hypothesis of Usher and Priestley,* the photo-synthesis of carbon dioxide and water is accompanied by the formation of hydrogen peroxide, and it is this latter compound that brings about the bleaching of the chlorophyll. From what we know of photo-chemical activity in other organic compounds, it would, however, not be unlikely that the rays of light absorbed by the chlorophyll may bring about a chemical change in it which is itself sufficient to initiate the series of chemical reactions resulting in the formation of sugar and starch. Thus Hoppe-Seyler,† quoted by Loeb,‡ "expressed the idea that chlorophyll undergoes first a combination with H_2CO_3 which, under the influence of light, falls apart in such a way as to yield chlorophyll (or the catalyser contained therein), O_2 and a third product, the latter being sugar or a substance from which sugar may be formed." "It is obvious," says Loeb, "that Hoppe-Seyler's idea represents that conception of the action of the catalyser which is more and more supported by the facts."

Hansen§ suggests that the chlorophyll is capable of forming an unstable compound with carbon dioxide, and that it is then passed on to the plasma of the chlorophyll grain to be converted into carbohydrate. Sir W. N. Hartley,‖ in discussing this, says that it is, however, much more probable on chemical grounds that the compound of chlorophyll with carbon dioxide is entirely decomposed, first by the elimination of oxygen, and, secondly, by the elimination of water, so that there are successively formed compounds of chlorophyll (1) with carbon dioxide; (2) with formic aldehyde; (3) with glucose; and, finally, starch, completely formed, is split off the molecule.

The Bleaching of Chlorophyll in Light.

Crude chlorophyll was obtained in the ordinary way by boiling leaves of grass or other plants in water and then extracting with alcohol. Methylated spirit may be used for this purpose, but it is more satisfactory to use absolute alcohol. In order to obtain the chlorophyll in as pure a state as possible, the strong alcoholic solution was first filtered, then evaporated to dryness, and dissolved in petroleum ether. For many experiments ordinary ether will serve, but for general use petroleum ether is to be preferred. Paper tinged with chlorophyll, either in alcoholic solution or in petroleum ether solution, was used, and also films of chlorophyll made by the evaporation of the chlorophyll solution on glass plates and in glass tubes and flasks.

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* 'Roy. Soc. Proc.,' vol. 77.

† 'Physiologische Chemie,' p. 139, Theil I (1877).

‡ 'Dynamics of Living Matter.'

§ 'Bied. Centr.,' 1888, see 'Chem. Soc. Journ.,' Abstracts, 1888.

‖ 'Chem. Soc. Journ.,' 1891.

The bleaching of chlorophyll can be conveniently demonstrated by exposing the half of a strip of paper tinged with chlorophyll to the light, the other half being kept in the dark. In sunlight the bleaching takes place very rapidly, but very slowly in diffused light.

The action of the different rays of the spectrum can be shown by exposing a piece of paper tinged with chlorophyll or a glass plate covered with a layer of chlorophyll to a sunlight spectrum, and it will be seen that the bleaching takes place as described in Herschell's experiments and more recently by Reinke* and by Dangeard† in those parts of the spectrum where the light is absorbed. A convenient method of showing the different effects of the principal parts of the spectrum is to make use of filters through which definite wave-lengths are transmitted. The Wratten and Wainwright filters are suitable for this purpose, and the action of light is much more rapid than with the pure spectrum. The disadvantage of filters is that the different colours absorb varying proportions of the light which they are supposed to transmit. Thus whilst a red filter may transmit 78 per cent. of the light, a blue filter may transmit only 16 per cent. of it.

The tricolour set of filters supplied by Messrs. Wratten and Wainwright divide the visible spectrum into three nearly equal parts—red, green, and blue—with some slight overlapping, but as this green allows rather more of the yellow and blue ends of the spectrum to pass than is desirable, it is better to add to the green another one which limits its range. The different parts of the spectrum transmitted through the three filters which I have used are as follows:—

Red—Standard tricolour filter, λ 710–590.
Green—Standard tricolour plus green (two filters), about λ 550–480.
Blue—Standard tricolour filter, λ 510–400.

The bleaching of chlorophyll takes place very rapidly through the red filter, much more slowly through the green and blue filters. If, however, the light is allowed to act for a longer time through the blue and green filters, the bleaching then becomes as pronounced through the blue as through the red filter. Thus in bright sunlight it takes approximately 8–10 times as long to bleach chlorophyll paper through the blue filter as through the red. This seems to indicate that the different effects of the red and blue ends of the spectrum are proportional to (1) the absorption of light, and (2) the energy coefficient of the different parts of the spectrum in which the absorption bands appear. Kniep and Minder* have pointed out that the effects produced in photo-synthesis are approximately proportional to the relative energy absorbed.

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* 'Bot. Zeit.,' 1885.

† 'Le Botaniste,' 1912 and 1913.

The Photo-decomposition Products of Chlorophyll.

The following experiments show that in the decomposition of chlorophyll by light two substances are produced, one giving the reactions of an aldehyde and the other an oxidising substance giving reactions with potassium iodide, by which the iodine is set free:—

Experiment 1: A piece of paper, tinged with alcoholic solution of chlorophyll, was arranged so that one-half of it was exposed to a good light, the other being kept dark. The half exposed to the light became bleached, and when placed in Schiff's solution the exposed portion developed a beautiful pink, the unexposed half remaining green with no pink coloration.

Experiment 2: If a piece of chlorophyll paper exposed to light as in Experiment 1 is placed in a solution of potassium iodide, the half exposed to the light becomes reddish-blue in colour, due to the liberation of the iodine, which acts upon the starch contained in the paper. The reddish-blue colour is probably due to the action of iodine upon starch in the presence of an excess of potassium iodide, for when the paper is washed in water the reddish-blue colour disappears and is replaced by the ordinary blue starch coloration.

Similar reactions to those described in these two experiments were found to take place when the paper itself was exposed to light without the chlorophyll, but the coloration was not so strong in either case.

Experiment 3: Two pieces of common note paper, similar to that used in Experiments 1 and 2, were exposed to light in the same way. One was placed in Schiff's solution. The exposed half became distinctly pink. The other was placed in potassium iodide and the exposed half became light brown. This seemed to indicate that the coloration in both cases was due to the paper and not to the chlorophyll.

Various kinds of paper were then experimented with, and it was found that in all cases a reaction both with Schiff's solution and with potassium iodide occurred, but that in the case of good superfine note paper the reactions were very slight. Accordingly, in all subsequent experiments with chlorophyll-tinged paper, a superfine note paper was used.

Experiment 4: A strip of W. H. Smith and Son's superfine cream laid note paper was tinged with chlorophyll and exposed to light as in Experiments 1 and 2. This was then cut longitudinally into two, and the two strips were then placed in Schiff's solution and in potassium iodide solution respectively. In both cases a strong reaction was obtained in those portions exposed to light. On comparing the results with the same paper not tinged with chlorophyll it was found that the reaction both in Schiff's solution and in potassium iodide solution was very strong with the chlorophyll-tinged paper but slight and almost negligible with the plain paper. Prolonged exposure of the plain paper to light gives a stronger reaction, but in no case as strong as the chlorophyll-tinged paper.

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* 'Zeit. Bot.,' 1909.

It was important to determine whether the solution of chlorophyll itself is able to give the reaction, apart from the paper. As alcohol gives a strong reaction with Schiff's solution it was necessary to dissolve the chlorophyll in some other solvent. For this purpose petroleum ether is suitable, as it does not give any reaction with Schiff's solution or with potassium iodide solution either in the dark or in the light.

Experiment 5: Four small test-tubes were partly filled with a solution of chlorophyll in petroleum ether and tightly corked. Two were exposed to the light and two kept in the dark. When those exposed to light were considerably decolorised, a small quantity of Schiff's solution was added to one and a small quantity of potassium iodide plus starch solution was added to the other. These solutions did not mix with the petroleum ether, but on shaking up the test-tubes the Schiff's solution became bright pink, the potassium iodide and starch solution became bluish-brown. The petroleum ether solutions which had been kept in the dark were treated in the same way with Schiff's solution and potassium iodide starch solution respectively, and in neither case was any reaction observed.

These experiments show clearly that the decomposition of chlorophyll is accompanied by the formation of an aldehyde and of a substance capable of oxidising the potassium iodide and setting free the iodine. It is extremely interesting to find that the same reactions are obtained with some kinds of paper when exposed to light. This is probably due to the decomposition of a substance in the paper the nature of which is unknown.

The same results are obtained when films of chlorophyll on glass are exposed to the light, and it can be further shown that the oxidising substance produced is a gas soluble in water.

Experiment 6: About 5 c.c. of a strong petroleum ether solution is carefully evaporated in a 50 c.c. flask so as to leave a thin film of chlorophyll on the sides and bottom of the flask. The ether should be completely evaporated, and a stream of air forced through the flask to remove all traces of the ether. The neck of the flask should be surrounded with black paper. A few drops of distilled water are placed in the flask. A strip of potassium iodide paper about two inches long is then attached to a cork, and the flask is corked up so as to allow the strip of paper to hang down in the neck of the flask. Another flask should be fitted up in precisely the same way but without chlorophyll. Both flasks should now be exposed to the sunlight. The bleaching of the chlorophyll takes place very rapidly. The strip of potassium iodide starch paper becomes purplish blue in the chlorophyll flask, showing that iodine has been liberated, but remains quite unchanged in the control flask. The strip of potassium iodide starch paper is now removed and a few more drops of distilled water are placed in the flask, which is then corked and the contents well shaken up. The water in the flask is then poured into two tubes. To one of these a few drops of Schiff's solution is added and a pink coloration soon develops, showing the presence of an aldehyde. To the second tube a few drops of a 10-per-cent. solution of potassium iodide is added, and then on the addition of a freshly made starch solution, a blue or reddish-blue coloration is obtained, indicating the presence of an oxidising agent capable of setting free the iodine in the potassium iodide.

If the bleaching has been continued long enough, the sides of the flask are now covered with a thin white layer of a substance which should be well washed to get rid of the remnants of the soluble aldehyde, and it will then be found that this white substance is insoluble in either hot or cold water. If, however, the bleaching is prolonged for a considerable time a much smaller amount of the insoluble white substance remains.

If we expose the chlorophyll paper behind coloured filters, we find that both the aldehyde reaction and the potassium iodide reaction are much stronger in the red than in the blue and weakest in the green. If, however, the exposure behind the green and blue filters is prolonged to about 8 or 10 times that of the red, the reaction in the blue becomes as strong as in the red.

The reaction for aldehyde is therefore proportional to the bleaching effect, and is approximately proportional therefore to the synthetic activity in the different parts of the spectrum.

The longer the light is allowed to act, the more completely does the chlorophyll become bleached, with a corresponding increase in the aldehyde reaction. In the case of the potassium iodide reaction, however, the converse is the case. When chlorophyll films, either on paper or on glass, are submitted to the prolonged action of light, the reaction with potassium iodide is much weakened, and may be completely absent. The explanation of this is probably that the oxidising substance is a volatile gaseous product, which tends to disappear as soon as it is formed, whilst the aldehyde is a solid which remains in the paper or in the film left on the glass. But it is not impossible that the oxidising substance may be of service in connection with the chemical reactions that take place in the chlorophyll, and may become used up in this way.

Instead of the extract of chlorophyll we may use dried leaves, or the chlorophyll expressed from living leaves and spread upon paper. We may also use layers of *Euglena viridis*, algæ and other green organisms spread over the surface of paper. In all these cases we can get by appropriate treatment, after exposure to light. both the aldehyde and potassium iodide reactions.

We can also show that both these reactions take place actually inside a leaf when the chlorophyll is sufficiently bleached. Thus if sunlight is condensed by means of a lens upon a living Oxalis leaf which contains abundance of starch, the chlorophyll in a small area of the leaf is bleached. If the leaf is now treated with Schiff's solution we get a strong aldehyde reaction in the bleached part ; if treated with potassium iodide solution the starch grains in and around the bleached area become coloured blue. The last experiment is not an easy one to perform as it is very difficult to hit just the right moment to stop the bleaching in order to get the potassium iodide reaction.

Is Formaldehyde produced by the Photo-decomposition of Chlorophyll?

The observations of Pollacci,[*] Usher and Priestley,[†] Harvey Gibson,[‡] and Schryver[§] all show that formaldehyde is produced when chlorophyll is exposed to sunlight in the presence of carbon dioxide but not in its absence, or possibly in minute quantities only. It is therefore important to determine whether the aldehyde produced in my experiments is composed of formaldehyde or whether it contains formaldehyde. The test used by Harvey Gibson gives a very pronounced reaction even when formaldehyde is present in quite small quantities. I have obtained a reliable reaction with 1/1,000,000, and a very pronounced reaction with 1/100,000. The test is carried out as follows:— About 3 c.c. of pure concentrated sulphuric acid are placed at the bottom of a small test-tube ; a few drops of a 5-per-cent. solution of gallic acid in absolute alcohol are poured gently on to the surface of the sulphuric acid and the liquid to be tested is then added ; if formaldehyde is present, a beautiful blue-green ring appears between the upper and lower liquids. Unfortunately this test is not reliable, as dilute solutions of sugar and starch and various other substances bring about the formation of a green or blue-green ring. This may be due to the fact that the sulphuric acid decomposes such substances as starch and sugar, and that a transitory product of this decomposition may be formaldehyde. Consequently, although the reaction is extremely useful for purposes of preliminary test, it cannot be relied upon to prove the presence of formaldehyde.

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[*] 'Inst. Bot. d. R. Univ. di Pavia,' 1902 ; see 'L'Année Biologique,' 1903.

[†] 'Roy. Soc. Proc.,' 1906.

[‡] 'Ann. Bot.,' 1907.

[§] 'Roy. Soc. Proc.,' 1909.

Rimini's test, as modified by Schryver, is extremely sensitive to formaldehyde and will easily detect 1/1,000,000. Here, however, the presence of various substances in the crude chlorophyll seems to interfere with the reaction, as shown by Schryver, and I have not been able to satisfy myself that the colour reaction given by this test with solutions of bleached chlorophyll is due to formaldehyde. Colour reactions are obtained which seem to indicate that formaldehyde is present in films exposed to light both in the presence and in the absence of carbon dioxide, but the reaction varies considerably with certain limits. Sometimes a dirty orange colour is produced, which is nothing like so distinct as the colour obtained with 1/1,000,000 of formaldehyde, sometimes a deeper coloration which more nearly resembles the formaldehyde coloration, but is more of an orange red or deep orange than the bright, clear red of the formaldehyde reaction. In any case, none of my experiments shows more than a very small quantity of formaldehyde in this way, although the reaction given by Schiff's test in all cases indicated a much larger amount of aldehyde. For example, a solution of bleached chlorophyll showed a reaction for aldehyde with Schiff's solution equal to more than 1/25,000, but on testing the same solution with Rimini's test, the result showed the presence of certainly not more than 1/1,000,000 of formaldehyde. It appears to me from a large number of experiments that, although the aldehyde in the bleached chlorophyll may contain a small quantity of formaldehyde, the major part of it consists of some other aldehyde, the nature of which I have not been able to determine.

The Oxidising Compound of Chlorophyll.

The gaseous oxidising substance formed on exposure of chlorophyll to light is soluble in water. The experiments of Usher and Priestley suggested the possibility that it might be hydrogen peroxide. A solution was prepared by exposing a film of chlorophyll on water to the light, which gave a strong reaction with potassium iodide and starch, the iodine being liberated at once and colouring the starch blue. Various well-known tests for hydrogen peroxide were then tried, but all gave a negative result. The following are some of the results obtained :—

Dilute solution of chromic acid with sulphuric acid. This gives a distinct blue coloration with 1/1,000,000 of hydrogen peroxide, but no reaction with the chlorophyll solution.

One of the most delicate tests for hydrogen peroxide appears to be that given by Roscoe and Schorlemmer in their text-book. When hydrogen peroxide is added to a solution of potassium iodide and ferrous sulphate, iodine is set free. Other oxidising agents have the power of liberating iodine from potassium iodide, but not in the presence of ferrous sulphate. I have obtained a distinct reaction with 1/50,000 of hydrogen peroxide and a reliable reaction with 1/1,000,000. In the presence of ferrous sulphate the chlorophyll derivative gives no reaction, although the same solution gave a strong reaction with potassium iodide and starch alone.

Leuchter's test*: With this I obtained a very clear reaction with 1/500 hydrogen peroxide, but no reaction with 1/50,000. No reaction was obtained with a bleached chlorophyll solution.

Titanium dioxide in concentrated sulphuric acid gives an orange-red colour with 1/5000 of hydrogen peroxide, and a distinct yellow colour with 1/50,000 ; no coloration was given with the chlorophyll solution.

Experiments were also tried with a solution containing ferric chloride and potassium ferricyanide. This gives a precipitate of Prussian blue with solutions of hydrogen peroxide ; solutions of the chlorophyll derivative only give a greenish yellow colour.

All these tests indicate, therefore, that the chlorophyll derivative is not hydrogen peroxide.

The experiment was then tried of exposing a film of chlorophyll in the dark to the action of a 20-per-cent. solution of hydrogen peroxide. If, as Usher and Priestley state, the decolorisation is brought about by hydrogen peroxide, we ought to get a very pronounced effect with so strong a solution. The experiment, however, showed that even after ten days' exposure to the hydrogen peroxide, the chlorophyll was far from completely bleached, and was still of a yellow or yellowish green colour. The experiment was tried many times in different ways, but always with the same result. The bleaching of chlorophyll in the light appears not to be due, therefore, to the action of hydrogen peroxide, and the most probable explanation seems to be that the light absorbed brings about a combination of the chlorophyll with oxygen resulting in the formation of an organic peroxide.

It is interesting to note that other colouring matters react to light in the same way with the formation of an oxidising substance capable of bringing about the liberation of iodine from potassium iodide. Thus, if strips of paper are soaked in solutions of the following dyes—methyl violet, methyl green, eosin, fuchsin, and fluorescein, and are then exposed to light and afterwards treated with a 10-per-cent. solution of potassium iodide, the iodine is liberated and the starch contained in the paper is coloured blue or reddish blue, a strong reaction being obtained in all cases. Cyanin, on the other hand, although readily bleached by the light, does not give this reaction.

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* 'Chem. Zeit.,' 1911, see 'Chem. Soc. Journ.,' Abstracts, 1911.

Experiments made with narrow glass tubes lined with a thin layer of methyl violet and eosin show, on exposure to light, that, during the process of bleaching, oxygen is used up, but this is not the case with cyanin, which becomes completely bleached without any appreciable rise of water in the tube. In the case of methyl green and eosin, the absorption of oxygen does not take place as rapidly and is not so pronounced as in the case of chlorophyll.

The Photo-decomposition of Chlorophyll in a Brown Sea-weed—Laminaria.

In order to make experiments on the chlorophyll contained in the brown sea-weeds, I collected pieces of fresh fronds of laminaria on the sea-shore and brought them home wrapped in pieces of ordinary white paper. On removing the paper, I found a blue coloration here and there where the paper had been in close contact with the fronds. It was obviously the blue starch coloration due to iodine. I at first thought that it might be due to chlorine,* possibly contained in the paper, acting upon an iodine compound in the sea-weed and causing the liberation of iodine. A statement in Pfeffer's 'Physiology,' however, led me to suspect that the coloration might be due to free iodine given off by the laminaria itself. To test this, I took some fresh pieces of a frond of laminaria and placed them in a dilute starch solution free from chlorine. The solution became coloured blue, showing quite clearly the presence of free iodine. The colour disappeared again in a very short time, much more rapidly, so it appeared to me, than it would have done in a starch solution coloured by an ordinary solution of iodine. I accordingly tried the experiment again, and found on comparing it with a starch solution coloured with iodine to the same depth of colour, that the laminaria solution lost its colour several hours before the other. This indicated that the iodine was taken up again by the laminaria from the starch solution, and it occurred to me that this might be due to the slime which is secreted by the laminaria and which was found in large quantities in the solution. I therefore placed equal quantities of a light blue iodine-coloured starch solution in two test-tubes. To one of these I added distilled water; to the other an equal quantity of the slimy liquid obtained by soaking pieces of the frond of laminaria in water. The colour disappeared at once, on shaking up, in the tube containing the slime, but not in the tube to which distilled water only had been added. I then placed equal quantities of a very dilute iodine solution (iodine in potassium iodide) in two test-tubes. To one I added, as before, distilled water, to the other an equal quantity of the slimy liquid from laminaria. These were then shaken and left to stand for a short time. Equal quantities of a dilute starch solution were then added to each, with the result that the blue coloration appeared in the tube to which distilled water had been added, but no coloration at all in the tube containing the slime.

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* Chlorine is used in the bleaching of paper.

These experiments show, therefore, that iodine is absorbed by the laminaria slime, probably forming an additive compound with it, and it appeared probable that a much more satisfactory iodine reaction would be obtained with laminaria if the frond were first of all freed from slime by washing well in water. I obtained the reaction very readily on a bright spring morning on the sea-shore by placing pieces of the fresh frond free from slime in contact with starch paper. The reaction is, however, very unequal; all parts of a frond are capable of giving it, but not necessarily at the same time. The most vigorous reaction was obtained in the growing region of the frond, the swollen portion where the frond joins the stipe. The reaction appears to be associated with those layers of cells which contain the chlorophyll and the brown colouring matter. Sections of the stipe and of the thicker portions of the frond, when placed in contact with starch paper, showed a blue layer all round where the paper had been in contact with the peripheral chlorophyll-containing cells, and sections from the thinner portions of the frond also gave the same result.

Pieces of the frond kept in sea-water in the dark gave no reaction, or at times a slight one; a strong reaction was obtained when the fronds had been exposed to a good light. The presence of the slime, however, may prevent the reaction. So long as any of the brown colouring matter is present, the iodine reaction may be given, but it is entirely absent in those parts of the frond which have lost the brown colouring matter and show a green colour. Whether the iodine reaction is associated with the brown colouring matter I cannot say, but if one half of a piece of frond is placed for a short time in hot water to destroy the brown coloration, the iodine reaction is obtained only with the brown portions of the frond.

It is probable, therefore, though not quite certain, that the action of light on the chlorophyll of laminaria brings about the production of an oxidising substance capable of effecting the decomposition of iodine compounds which may be contained in the chlorophyll cells of the plant, and that the iodine thus set free may either escape or be re-absorbed by the slime which occurs in such abundance in laminaria.

The Photo-decomposition of Chlorophyll takes place only in the Presence of Oxygen.

Three test-tubes were taken and lined with a film of chlorophyll by the careful evaporation of a petroleum ether solution of grass chlorophyll. One was placed with its open end in a strong solution of potassium hydrate and pyrogallol, to absorb the oxygen; the second was placed in a strong solution of potassium hydrate to absorb the carbon dioxide, and the third was placed in distilled water. All three were kept in the dark for 24 hours, and were then exposed to the light. The second and third bleached very rapidly. The first, which contained no free oxygen, remained unbleached even after an exposure of some months. At the time of writing, it has been exposed for four months in a south window and is still unbleached. The second and third gave strong reactions both for aldehyde and for the oxidising agent. The experiment shows quite clearly that the bleaching of chlorophyll is the result of oxidation brought about under the influence of light.

If this is correct, it occurred to me that we ought to be able to show that oxygen is absorbed in the process. To test this, I obtained four tubes 16 cm. long and 4 mm. in diameter; they were drawn out at one end to a point, which was then broken off, so as to leave an opening less than 1 mm. in diameter. Three of these were lined with chlorophyll from a petroleum ether solution. Care was taken to get rid of all traces of the ether by forcing a stream of air through the tubes for some time. I found a bicycle pump useful for the purpose. The fourth tube contained no chlorophyll and was simply used as a control. The three chlorophyll tubes and the empty tube (No. 4) were then placed with their open ends downwards in distilled water contained in two separate beakers. The water was at a sufficient depth to allow of its entry into the tubes to a height of 2 cm. This was done to allow of the expansion of the air in the tubes when they were exposed to the heat of the sun. The upper narrow ends of the tubes were then sealed with the bunsen flame. They were all placed in the dark for 12 hours. The next day they were all carefully measured, and it was found that the water was at the same height in each tube. One of the chlorophyll tubes and the control tube were then exposed to sunlight. They were kept under careful observation to see that the expansion inside the tubes did not drive out any of the air. The chlorophyll in the chlorophyll tube soon began to bleach, and the level of the water began to rise and, in the course of a few hours, it reached a height of rather more than 1/5th of the tube, showing that part of the air, probably the oxygen, had been absorbed. The water in the control tube did not rise. The tubes were allowed to remain in the light until no further rise in the chlorophyll tube took place. At this stage the chlorophyll was not completely bleached, but as on prolonged exposure to sunlight no further bleaching took place, it seemed fair to conclude that all the oxygen had been used up. The tubes were now brought to the back of the room into diffused light and allowed to stand for some hours. Careful measurement of the chlorophyll tube showed that the level of water in the tube had risen to a height corresponding exactly to the percentage of oxygen likely to be present in the air enclosed in the tube when the experiment started. To test this, the control tube was placed in a strong solution of potassium hydrate and pyro. This gradually diffused into the water contained in the tube and then gradually absorbed the oxygen in the tube. In the course of 24 hours, the level of this solution had risen in the tube until it was stationary, and this was found to be almost exactly the same height as the level of the water in the chlorophyll tube. This showed pretty conclusively that in the bleaching of the chlorophyll the whole of the oxygen of the air had been used up. To show that no oxygen was left, however, the chlorophyll tube was itself placed in the potassium hydrate pyro solution; the tube was gently warmed until the water was nearly driven out, and then, on cooling, the pyro solution entered the tube and rose to exactly the same level as the level of the water previously contained in the tube, and remained at that level, thus showing that no oxygen had been left in the tube.

Experiments were then made with the other two tubes which had been kept in the dark; one was placed in a solution of potassium hydrate and pyro, the other was placed in the sunlight for some hours. On leaving them to stand for some hours at the back of the room to equalise the temperature, the level of the liquid in both was the same. Further experiments conducted more carefully with due attention to the corrections necessary for temperature and pressure showed quite conclusively that oxygen is absorbed when chlorophyll is bleached in the light, and that if sufficient chlorophyll is present, the whole of the oxygen in the air in contact with it is used up. It is possible, in fact, to make use of chlorophyll instead of pyrogallol and potassium hydrate in making quantitative determinations of the amount of oxygen contained in the air.

The Presence of Carbon Dioxide is not Necessary for the Photo-decomposition of Chlorophyll.

The changes described in the last section are brought about just as rapidly and as completely in the absence of carbon dioxide as when it is present.

Thus, two flasks were prepared with films of chlorophyll from a petroleum ether solution of grass chlorophyll, as nearly as possible similar to each other. Into one 2 c.c. of distilled water were placed together with a short tube containing a stick of potassium hydrate to absorb carbon dioxide, and then tightly corked with a strip of potassium iodide starch paper in the neck of the flask. Into the second flask was placed 2 c.c. of water containing carbon dioxide from a sparklet apparatus, and this was then corked up, also with a strip of potassium iodide starch paper. Both were then exposed to sunlight, and it was found that the bleaching was equally rapid in both cases, that the potassium iodide starch paper was discoloured in both to the same extent, and that the aldehyde in both was similar in amount, so far as could be judged by the depth of colour produced with Schiff's solution. The experiment was tried several times under varying conditions, but the result was always the same, the presence or absence of carbon dioxide made no difference in the effects produced by the light.

But although carbon dioxide is not necessary for the photo-decomposition of chlorophyll, it is possible that, when present, it may be used up in some way corresponding to the photo-synthesis in the living plant. Experiments conducted with known quantities of carbon dioxide in contact with thin chlorophyll films in long narrow tubes, as used in previous experiments, showed, however, that, whether present in large or in small quantities, the carbon dioxide is apparently not used up in the bleaching of chlorophyll outside the plant. The bleaching takes place quite readily so long as oxygen is present, but the subsequent tests showed no diminution in the amount of carbon dioxide, so far as this could be measured with caustic potash.

The experiments are not conclusive, however. It is possible that a very small amount of carbon dioxide, too small to be measured quantitatively by the somewhat rough methods at my disposal, may be used up, but the fact that carbon dioxide is certainly not necessary for the bleaching of chlorophyll or the production of aldehyde, and that, so far as my experiments go, no appreciable amount of carbon dioxide is used up even when present in considerable quantities, would seem to indicate that, under the conditions of my experiments, carbon dioxide is not used up by the chlorophyll when bleached in the light. It is important, however, that further experiments should be made in which the carbon dioxide determinations can be made more accurately.

The Photo-decomposition of the Green and Yellow Pigments of Chlorophyll.

The green and yellow pigments were obtained by shaking up an alcoholic solution of grass chlorophyll with benzene. The alcoholic solution of the yellow pigment was then evaporated to dryness and extracted with petroleum ether. The benzene solution of the green pigment was treated in the same way. Thin films of these two colouring matters were then exposed to light (*a*) in the absence and (*b*) in the presence of carbon dioxide. In both cases the yellow pigment bleached rapidly, and gave a very strong reaction both with Schiff's solution and with potassium iodide. The green pigment bleached much more slowly and did not give quite as strong a reaction with either Schiff's solution or potassium iodide. Similar results were obtained with strips of paper tinged with the green and yellow pigments respectively. Thus, paper tinged with yellow pigment from grass chlorophyll gave, after 40 minutes' exposure to diffuse sunlight in January, a strong reaction both with Schiff's solution and potassium iodide. The green pigment under the same conditions gave no reaction. Paper tinged with ordinary grass chlorophyll gave a slightly stronger reaction than the yellow pigment. At the end of two hours the green pigment gave a very slight reaction with Schiff's solution, but a strong reaction with potassium iodide, the yellow pigment a strong reaction in both cases.

The more rapid oxidation of the yellow pigment can also be seen by lining narrow glass tubes (*a*) with the yellow and (*b*) with the green pigment. These are then placed with their open ends downwards in water and exposed to bright sunlight. The water rises very rapidly in the tube with the yellow pigment, showing a rapid absorption of the oxygen, but more slowly in the tube with the green pigment. In both cases, however, the whole of the oxygen in the tube ultimately becomes used up, and the water rises to the same level in each.

The tubes were 39·4 cm. long. After exposure to light the water rose 8·6 cm. The height of the water in a control tube of the same length was 0·5 cm. Consequently on subtracting this both from 39·4 and 8·6, the ratio 8·1 to 38·9 gives 20·82 as the percentage of oxygen absorbed.

The Action of Oxidising Agents upon Chlorophyll.

As the decomposition of chlorophyll by light appears to be an oxidation process brought about by the oxygen of the air in the presence of light, it occurred to me that similar effects might be brought about in the dark by the use of some of the ordinary agents such as hydrogen peroxide and permanganate of potash. I accordingly placed chlorophyll films, obtained by the evaporation of a petroleum ether solution of grass chlorophyll, in contact with a very dilute solution (pink) of permanganate of potash. These were allowed to act for six days and were then examined. In all cases the chlorophyll films showed considerable bleaching, and on carefully washing them with water to get rid of the oxidising agents and then bringing them into contact with Schiff's solution, a pronounced pink coloration was produced, showing the presence of an aldehyde. The aldehyde at first appeared in the film, but the colour soon became dissolved in the Schiff's solution, leaving a thin white layer in the glass. The powerful oxidising solution made by adding a few drops of sulphuric acid to a dilute solution of permanganate of potash acts very rapidly in bringing about the oxidation of chlorophyll and the production of an aldehyde. A film of chlorophyll placed in contact with the solution began to bleach at once, and in half an hour gave a very pronounced reaction with Schiff's solution.

The following experiments were also tried: A film of grass chlorophyll placed in the dark in contact with a 20-per-cent. solution of hydrogen peroxide for 16 days and then washed in water gave a strong reaction with Schiff's solution. The pink colour was first of all developed in the film, but soon became washed out in the solution, leaving a whitish layer on the glass.

A film of the yellow colouring matter of chlorophyll was treated in the same way, and gave a similar reaction with Schiff's solution.

A film of the green colouring matter of chlorophyll, treated in the same way, showed very slight decoloration or bleaching, and gave no reaction with Schiff's solution.

Similar results were obtained when strips of paper tinged with chlorophyll were used.

The bleaching of chlorophyll in the presence of hydrogen peroxide takes place much more rapidly in the light than in the dark. Thus, a film of grass chlorophyll was completely bleached in 12 hours in the light, but a similar film was hardly changed after ten days in hydrogen peroxide in the dark. In bright sunlight, complete bleaching was effected in three hours.

The film which had been bleached in the light gave a very strong reaction for aldehyde, and the oxidation of the film was so complete that only a trace of white film was left on the glass after the aldehyde had been dissolved out by the Schiff's solution.

The dilute sulphuric acid solution of permanganate of potash is a much more powerful oxidising agent than hydrogen peroxide in its action upon chlorophyll. In the dark a very pronounced bleaching is obtained in half an hour, and nearly complete decolorisation is effected in about two hours, with a correspondingly strong reaction for aldehyde. In the light the bleaching takes place slightly more rapidly than in the dark.

The yellow colouring matter of chlorophyll bleaches very rapidly, the green colouring matter very slowly in permanganate of potash and sulphuric acid. Two films of equal size were prepared in two test-tubes, (1) of the yellow colouring matter, and (2) of the green colouring matter of grass chlorophyll, and equal quantities of the permanganate solution were poured into each with the following results:—

(1) Yellow colouring matter: In 50 seconds the permanganate solution was nearly colourless. At the end of two minutes it was poured off; the film was quite bleached and gave a strong reaction for aldehyde with Schiff's solution. The experiment was repeated with the yellow pigment from leaves of *Chrysanthemum parthenium* (Feverfew) with a similar result.

(2) Green colouring matter: At the end of one hour the permanganate solution was not quite colourless; the film still showed a yellow-green coloration, but was more strongly bleached in the thinner parts. In order to ensure more complete bleaching, fresh quantities of permanganate solution were added from time to time, but even at the end of 12 hours the bleaching was not complete. On testing with Schiff's solution, the film gave, however, a strong reaction for aldehyde.

None of the films bleached by oxidising agents, either in the dark or in the light, gave a reaction with potassium iodide.

Experiments with strips of paper tinged with (1) grass chlorophyll, (2) the yellow pigment, and (3) the green pigment from grass chlorophyll, gave similar results on treatment with the permanganate solution. (1) and (2) began to bleach at once, and at the end of half an hour gave a strong reaction for aldehyde; (3) showed a slight reaction only at the end of two hours.

From these experiments we may draw the extremely interesting conclusions that, so far as the production of an aldehyde is concerned, the oxidation of chlorophyll in the dark by means of solutions of hydrogen peroxide and permanganate of potash brings about a similar change to that which is effected when chlorophyll is acted upon by light in the presence of oxygen.

We have seen that the yellow colouring matter obtained both from grass chlorophyll and from the chlorophyll extracted from the leaves of *Chrysanthemum parthenium* bleaches very readily in the light, and also in oxidising agents. This led me to suspect that the yellow colouring matter extracted from etiolated leaves—leaves in which only a yellow colouring matter had developed—would give the same results. The yellow-orange colouring matter extracted from etiolated rhubarb leaves was found, however, to bleach more slowly, both in light and in oxidising reagents, than ordinary chlorophyll obtained from grass, and very much more slowly than the yellow pigment from grass chlorophyll. Whether this has anything to do with the lack of photo-synthetic activity which Miss Irving* has found in chlorophyll not completely developed I cannot say, but, considered in the light of Miss Irving's observations, that the photo-synthetic activity of chlorophyll does not reach its full strength until the chlorophyll has been fully formed, the retardation of the photo-oxidation of the etiolin is of considerable interest.

It is, of course, possible that the yellow colouring matters from other plants may be found to behave differently in this respect, and too much stress must not be laid, therefore, upon the experiments just described. It is proposed to continue these observations.

The Action of Reducing Agents upon Oxidised Chlorophyll.

We have seen that the photo-oxidation of chlorophyll results in the production of an oxidising substance and of an aldehyde. Both are therefore oxidation products, and it was of some interest to ascertain the action of reducing agents upon them.

Three strips of paper coloured green by grass chlorophyll in a petroleum ether solution were exposed to the light until visibly bleached: 1 and 2 were then placed in a strong solution of phenylhydrazine, 3 was cut in two and one portion was treated with Schiff's solution, the other with a 10-per-cent. solution of potassium iodide. Both gave a strong reaction. After being kept in the phenylhydrazine solution for three hours, 1 was placed in Schiff's solution, 2 in potassium iodide solution, and in neither case was any reaction obtained.

Similar results were obtained with stannous chloride, and with a pyro-soda photographic developer.

Chlorophyll paper oxidised in the dark by the permanganate of potash solution, then treated for three hours with phenylhydrazine hydrochloride also gave no reaction either with Schiff's solution or potassium iodide.

The reducing agents do not bring back the green colour to the oxidised film, but the activity of both the products of chlorophyll photo-oxidation is destroyed.

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* 'Ann. Bot.,' 1910.

Conclusion.

The experiments outlined in this paper indicate, so far as experiments conducted on dead chlorophyll extracts can be taken as an indication of what goes on in the living plant, that the bleaching of chlorophyll is not a result of the activities set up by photo-synthesis, as suggested by Usher and Priestley, but is the actual basis and starting point of the changes set up in the green leaf under the influence of light. In other words, the aldehyde produced under the conditions described in this paper is a product of the photo-decomposition or photo-oxidation of chlorophyll and is not a result of the direct photo-synthesis of carbon dioxide and water.

The aldehyde appears to be in fact purely a product of the photo-oxidation of chlorophyll. This modifies our conception of the changes which may possibly take place in the living plant. We know that carbon dioxide is necessary for the production of sugar and starch in the living cell. But if the sugar and starch are produced as the result of changes taking place in an aldehyde, and if the aldehyde is a direct product of the decomposition of chlorophyll, then we must conclude that the carbon dioxide before it can be used is built up independently into the chlorophyll molecule, and it is possible that the production of sugars and starch may be initiated by photo-oxidation of the chlorophyll rather than by the direct photo-synthesis of carbon dioxide and water.

Summary.

1. An account is given in this paper of some of the effects produced by light upon chlorophyll. When chlorophyll is exposed to the light at least two substances are formed, one of which is an aldehyde or mixture of aldehydes and the other an active chemical agent, capable of bringing about the liberation of iodine from potassium iodide.

2. These products of decomposition can be very easily demonstrated by means of strips of paper tinged with chlorophyll. When bleached in the light and placed in Schiff's solution, a deep pink colour is developed showing the presence of an aldehyde; but if placed in a 10-per-cent. solution of potassium iodide, a reddish blue coloration, which becomes blue on washing in water, is developed, showing the presence of an oxidising agent. The same products are obtained when films of chlorophyll on glass are bleached in the light.

3. The bleaching of chlorophyll is less at the blue end of the spectrum than at the red end, with a corresponding variation both in the aldehyde and potassium iodide reactions. But if the exposure to the blue light is prolonged to about eight or ten times that of the red light, the reactions are just as pronounced. The bleaching and the corresponding products of decomposition

are probably therefore proportional to the photo-synthetic activity of the chlorophyll in the different parts of the spectrum.

4. The presence of formaldehyde is not very clearly indicated in my experiments. Rimini's test, as modified by Schryver, gives indications of a trace of formaldehyde when chlorophyll is exposed to light both in the presence and in the absence of carbon dioxide, but I do not consider the results reliable, and in any case the reaction given is nothing like so strong as is indicated by Schiff's solution. The test used by Harvey Gibson is also very sensitive to formaldehyde, but is unreliable as it gives a pronounced reaction with solutions of sugar and starch and other substances. All that can be said at present is that in the photo-decomposition of chlorophyll a considerable quantity of aldehyde is formed, with possibly a small amount of formaldehyde.

5. The oxidising substance appears not to be hydrogen peroxide, but it may be an organic peroxide derivative of the chlorophyll.

6. The bleaching of chlorophyll *in situ* in dead green leaves, algæ, and other chlorophyll-containing organisms, gives the same products as the chlorophyll extracts outside the plant.

7. If a fresh green leaf of *Oxalis acetosella* is exposed to an intense light concentrated upon it by a lens, as in Pringsheim's experiments, the bleached chlorophyll gives an aldehyde reaction when placed in Schiff's solution. If the leaf contains abundance of starch, it may, after the action of the intense sunlight, be placed in a solution of potassium iodide, when the oxidising agent set free from the chlorophyll will liberate the iodine, and the starch grains will be coloured blue. The experiment is not an easy one to perform, as it is so very difficult to hit just the right moment to stop the bleaching, in order to get the potassium iodide reaction. See also the experiments on Laminaria.

8. The decomposition of chlorophyll with the production of aldehyde and peroxide takes place just as readily in the absence of carbon dioxide as when carbon dioxide is present. My experiments show that carbon dioxide is not used up in the process even when present in considerable quantities. Carbon dioxide is not necessary therefore to the production of the aldehyde.

9. The photo-decomposition of chlorophyll takes place only in the presence of oxygen. Oxygen is used up in the process. If sufficient chlorophyll is present, all the oxygen in the air in contact with the chlorophyll is absorbed. Chlorophyll may be used instead of caustic potash and pyrogallol in the analysis of air.

10. Chlorophyll is slowly oxidised in the dark by a solution of hydrogen peroxide. In the light the action is more rapid, but not more so than when light acts on chlorophyll in the presence of oxygen. A rapid oxidation of

the chlorophyll takes place in the dark in the presence of a dilute solution of permanganate of potash to which a few drops of sulphuric acid have been added. In both cases an aldehyde is produced which can be made evident by means of Schiff's solution.

11. If a strip of potassium iodide starch paper is exposed to light under coloured filters the paper turns reddish blue under the blue filter, showing the liberation of iodine, but not under the red filter. If, however, the iodised starch paper is first of all tinged with chlorophyll and then exposed to light under the same filters, a strong reaction takes place under the red filter.

A strip of bleached chlorophyll paper, placed in contact with a strip of damp iodised starch paper in the dark, is also capable of effecting the liberation of iodine, and the starch paper turns blue.

12. It is suggested in conclusion that the production of sugars and starch in the green leaf may be initiated by the photo-oxidation of chlorophyll and the subsequent polymerisation of the aldehyde thus formed, rather than by the direct photo-synthesis of carbon dioxide and water.

Intermittent Vision.

By A. Mallock, F.R.S.

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Studies in Brownian Movement. I.—*On the Brownian Movement of the Spores of Bacteria.*

By John H. Shaxby, B.Sc., and E. Emrys-Roberts, M.D.

(Communicated by Principal E. H. Griffiths, F.R.S. Received November 19, 1913,—Read January 29, 1914.)

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The Controlling Influence of Carbon Dioxide in the Maturation, Dormancy, and Germination of Seeds.—Part I.

By Franklin Kidd, B.A., Fellow of St. John's College, Cambridge.

(Communicated by Dr. F. F. Blackman, F.R.S. Received January 10,—
Read March 5, 1914.)

Introduction.

The cause or causes conditioning arrested development in moist seeds and the nature of the impetus which results in germination are still in most respects obscure. The problem of the non-germination of maturing seeds while still upon the parent plant and the large range of cases of delayed or non-germination of shed seeds which to all appearances are in good conditions for germination form the basis of this research.

It is to be emphasised that the problem of seed dormancy is not limited to the case of the dry seed. The more important, but less obvious, conditions of dormancy are those found in moist maturing seeds, and in cases of delayed germination in the presence of sufficient conditions of moisture and temperature. It is these which have the most interesting analogies in other fields, and an analysis of which may be more fruitful from the point of view of physiology in general.

It is useful at the outset to examine certain conclusions that are being reached by workers who have set themselves to elucidate the processes of similar phenomena in other departments of physiology. In certain aspects, the latency of the unfertilised ovum offers an analogy with the latency of moist seeds. In each case the latency is only ended by the onset of definite causes; in each case in the absence of these causes the period of latency is sooner or later terminated by death; and in each case also the sequence of changes that follow the onset of the stimulus is, in a broad sense, physiologically comparable. The interest of this analogy, moreover, is increased by the prominence which has recently been given to a simple interpretation of the nature of the fertilisation stimulus. Loeb (1) has attempted to outline its essential features as follows. These appear to be, firstly, an acceleration of oxidations which follows destruction by cytolytic agents of a cortical layer in the egg which has hitherto prevented oxygen from reaching the surface of the egg and from penetrating into the latter sufficiently rapidly. Secondly, Loeb believes that an internal change takes place which renders innocuous the toxic products of oxidation. He shows that the unfertilised matured egg dies soon, and he attributes this to the toxic action of products of oxidation, as its life can be prolonged in the absence of oxygen.

Again, it has been a feature of recent work under many aspects to emphasise the action of the ordinary metabolic products of cell life in producing deep functional changes, both normal and abnormal. The nature of the action of these products is being studied in detail, and it has become clear in certain cases that what appears to be an act of excitatory stimulus producing a certain forward change is in reality the removal of a depressant stimulus normally present which acts as an inhibitant. Thus, for example, it has been recently shown* that the growth of the mammary glands in a pregnant female is due to a product of fœtal growth which acts by overcoming the inhibitory action of a substance which is normally present and prevents the development of these parts.

The case of antithrombin normally present in the blood in sufficient quantities to inhibit the action of any thrombin ferment formed, and so preventing any intervascular clotting, is well known. The study of immunity affords a very large number of instances of antibodies whose function is the inhibition of the harmful stimulation of poisons. Czapek (3) in his work on the anti-ferment reaction in tropistic movements of plants has added another interesting example in this line of discovery. He demonstrates geotropic stimulation to be accompanied by an accumulation of homogentisinic acid due to the action of an antiferment inhibiting its break-down by oxydase normally present.

In this paper the indicated problem of the dormancy of moist seeds has been attacked from the point of view that dormancy must be conditioned by the absence of an essential stimulus or by the presence of an inhibitory agent. The two-sided question therefore which is presented at the outset is as follows: What is the nature of the positive stimulus to germination or what is the nature of the inhibition which must be overcome to initiate this process?

Influence of Carbon Dioxide in Inhibiting the Germination of Moist Seeds.

(a) *Carbon Dioxide Inhibits the Germination of Seeds without Producing Injury.*—It will be useful to begin with a brief examination of the group of phenomena classed under the term "delayed germination." In one class of cases it is known that many seeds do not immediately germinate in nature even when to all appearance placed in optimum germinating conditions. This is true of a number of native species which remain in the ground during the winter, although freely germinating in the following spring. In another considerable class of cases the seeds appear to be capable of remaining indefinitely in the ground without germinating, while preserving latent their power of growth under certain conditions, the nature of which does not appear to be clearly understood. We find the embryos of these latent seeds to be apparently in good germinating conditions, that is, supplied with sufficient water, in an atmosphere containing the normal percentage of oxygen, and at a temperature sufficient for germination.

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* By Prof. Starling and Miss E. Lane-Claypon (2).

In a large number of cases of this phenomenon quoted by Nobbe and Hänlein (6), sporadic germination over periods of months, and even years, is a marked feature. In natural conditions *Brassica nigra* is an example of these cases of delayed germination. In Sussex it is locally called Kelke, and every farmer and labourer along the northern slope of the South Downs will give examples from his experience of the seeds sprouting in newly ploughed land after they have lain dormant for years, while the land has been under pasture or hay.

In certain of these cases of delayed germination in germinating conditions, non-germination has been shown by Ewart to be accompanied by a lack of water in the embryo due to the impermeability of the testa to water. These cases do not bear upon our problem. It is with the range of cases in which a full water supply is demonstrated that interest lies. So far as explanations based on experiment have hitherto been forthcoming for non-germination in these seeds, they have been mainly directed to elucidate this somewhat striking phenomenon from the point of view that the testa is shielding the embryo from a sufficient supply of oxygen.

Crocker (5) has reached this conclusion from his work upon the upper seeds of Xanthium burrs, which normally do not germinate till after they have lain over one year in the soil. He found that while at a temperature of 19° C. these seeds would not germinate—though containing a sufficient supply of H_2O and though lying in a normal atmosphere (*i.e.* with a partial pressure of oxygen equal to 150 mm.)—germination could nevertheless be immediately induced by removal of the testas. Recently Shull (4), working upon these same seeds, has given us the actual minimum values of oxygen necessary for the germination of the naked embryos. At a temperature of 21° C. the minimum partial oxygen pressure required by them is not more than 12 mm. If we are to adopt Crocker's view, therefore, that the non-germination of these seeds with the testa intact is due simply to the fact that only a subminimal quantity of oxygen can reach the embryo, we shall have to say that the wet testa is able to reduce the pressure of oxygen in its passage through it from 150 mm. pressure to less than 12 mm.

It is conceivable that in the maturation of the seed and in delayed germination under ordinary and special circumstances we may be dealing not with an insufficient oxygen stimulus but with an inhibitory cause or group of causes. Such a condition might result in the case of the seed if the testa acts in any way as limiting the aëration of the embryo, for we might expect then two results:—

(1) A reduction in the amount of oxygen reaching the embryo, and
(2) A relative rise in the actual CO_2 pressure in the embryo tissues.

The crucial question first arises, therefore, as to the actual effect of increased pressures of CO_2 in the tissues of the embryo. The experiments which follow have been immediately directed to ascertain in the first place the actual effect of increased pressures of CO_2 upon the germination of quickly germinating seeds.

Technique of Experiments made to Ascertain the Effect of Increased Partial Pressures of CO_2 *on Germination.*—In setting these experiments a known quantity of pure silica sand was first introduced into large flasks and saturated with water. This was done by adding an excess of water and then drawing it off by tipping the flasks. If this was carefully done the sand was left saturated with water in a layer adhering to the bottom of the flasks. The seeds were carefully dropped on to this surface by means of a glass tube, and, where necessary, as in the case of larger seeds, a further measured quantity of H_2O was added. The flasks were then stoppered with new rubber corks fitted with one glass tube closed by means of pressure tubing and a pinchcock. Gases in any proportion desired were now quickly introduced by first withdrawing a quantity of air by an air pump, the amount being read by a pressure gauge. Where small quantities of CO_2 were desired up to 6 per cent. of an atmosphere, the operation was performed by means of a specially made apparatus on the model of Hempel's gas burette, using mercury. By means of this apparatus very accurately measured amounts of air can be withdrawn and equal lots of CO_2 introduced. For higher percentages of CO_2 the air pump was employed. The artificial atmospheres were for the most part checked by analysis after setting. The carbon dioxide, oxygen, and nitrogen employed were in all cases from cylinders as supplied by the Carbonic Acid Company and British Oxygen Company.

Table I.—The Effect of Increased Partial Pressures of CO_2 on Barley (*Hordeum vulgare*) in Retarding and Inhibiting Germination, and the Resumption of Normal Activity on Removal of these Increased Partial Pressures.

Actual percentage of CO_2 in air as set. (By analysis.)	Germinations.							Final total percentage of germination.
	In presence of raised CO_2 pressures. After			In air after removal of raised CO_2 pressures. After				
	42 hrs.	70 hrs.	118 hrs.	20 hrs.	44 hrs.	70 hrs.	Final 12 days.	
0 (air with KOH)	7	9	10					100
6·0	7	8	9					90
12·0	9	9	9					90
17·3	5	8	8					80
23·5	3	6	6	8	9	9	9	90
29·5	3	3	5	8	9	9	9	90
35·0			1	8	10	10	10	100
37·5				5	7	8	8	80
43·5				1	2	3	6	60
96·0								0

Temperature, 20° C. thermostat. 10 seeds in each experiment.

Table II.—The Effect of Increased Partial Pressures of CO_2 on Peas (*Pisum sativum*) in Retarding and Inhibiting Germinations and the Resumption of Normal Activity on the Removal of these Partial Pressures.

Approximate percentage of CO_2 in air as set. (By analysis.)	Germinations.								Final total germinations out of five seeds in each case.
	In presence of raised pressures of CO_2. After				In air after removal of raised pressures of CO_2. After				
	44 hrs.	68 hrs.	96 hrs.	7th day.	8th day.	9th day.	11th day.	20th day.	
0 (air)	1	1	5	5					5
6	0	2	3	4					4
12	1	3	4	5					5
18		1	5	5					5
24		0	1	5					5
30		2	4	4	5	5	5	5	5
50					3	4	5	5	5
70					2	4	4	4	4
100					0	1	2	3	3

Temperature, 20° C. thermostat. Five seeds in each case.

Table III.—The Effect of Increased Partial Pressures of CO_2 on Bean (*Vicia faba*), Cabbage (*Brassica oleracea*), and Onion (*Allium cepa*) Seeds in Inhibiting Germination, and the Resumption of Normal Activity on the Removal of these Increased Partial Pressures.

Species of seed used.	Percentage of CO_2 in air in which seeds were set to germinate.	Time during which seeds remain in artificial atmosphere containing raised percentage of CO_2.	Resulting germination in artificial atmosphere containing raised percentage of CO_2.	Final percentages subsequently in normal air.	
				Of germinations.	Of good plants.
		days			
Cabbage (50 seeds)	25	10	All inhibited	72	72
	38	10	,,	88	88
	44	10	,,	76	76
	0 (air with KOH)	0	Normal germination at once	84	84
Beans (30 seeds)	45	8	All inhibited	95	85
	53	8	,,	75	55
	89	8	,,	85	50
	0 (air with KOH)	0	Normal germination at once	95	85
Onion (50 seeds)	23	11	28 per cent. germinated	44	44
	30	11	All inhibited	50	50
	68·7	11	,,	46	46
	0 (air with KOH)	0	Normal germination at once	60	60

Average temperature, 17·5° C.

(b) *The Peculiar Case of White Mustard* (Brassica alba).—*Brassica alba* was peculiar among the seeds experimented on, in that inhibition was continued indefinitely after the removal of the seeds from increased partial pressures of CO_2 to normal air, and was then only terminated by the treatments described in Tables IV and V.

White mustard seeds that have been inhibited by the action of CO_2 while germinating will lie indefinitely in germinating conditions without sprouting or with sporadic sprouting over long intervals. They have all the appearance of continued vitality, and they do not become attacked by moulds. The part played by the testa in securing the continuance of the inhibitory effect of carbon dioxide after the removal of the inhibitory agent is of great interest.

In the following table it will be seen that dormancy produced by CO_2 was continued for two to three months after removal of the seeds to air, suitable conditions for germination being maintained throughout. Finally the seeds returned to normal activity and germinated 100 per cent. in every case following the treatments described.

Table IV.—The Effect of Increased Partial Pressures of Carbon Dioxide on White Mustard Seeds (*Brassica alba*) in Retarding and Inhibiting Germination, the Continued Dormancy of the Seeds after their Removal to Air and their Final Resumption of Normal Activity following Certain Definite Treatments.

Approximate percentages of carbon dioxide in air as set.	Per-centage of CO_2 by analysis after 3 days.	Germinations while in presence of raised CO_2 pressures after						Germinations in air after removal of raised CO_2 pressures.									Number of seeds which had failed to germinate at all up to this time.	Final treatment of non-germinating seeds.	Number of germinations after this final treatment.	Final total per-centage of germinations.
								Seeds previously in CO_2 pressures from 9 to 15 per cent. for 72 hours.					Seeds previously in CO_2 pressures above 15 per cent. for 8 weeks.							
		18	28	36	48	60	72	12	32	36	48	96	4	8	12	∞				
		hours from beginning.						hours after removal to air.					days after removal to air.							
0 (Air)	1	7	18	20	20	20	20										0			100
3	3·6	2	16	19	20	20	20										0			100
6	6·4	1	14	17	20	20	20										0			100
9	9·1		3	7	15	17	18	18	19	19	20	20*					0			100
12	12			3	11	11	12	12	14	16	18	19*					1	testas removed	1	100
15	15·5			1	2	2	2	3	7	10	14	15*					4	” ”	4	100
18	17·6						2†						4	5	13	13*	7	dried for 3 weeks	7	100
21	20·8						0†						2	5	7	9*	11	” ”	11	100
24	23·2						0†						1	1	6	6*	14	” ”	14	100

Average temperature, 17·5° C.

* Germinating conditions in air maintained for 4 weeks with no further germinations resulting.

† CO_2 pressures maintained for 8 weeks with no further germinations resulting.

Table V.—A Summary of Experiments showing the Indefinite Suspension, even after Removal of Inhibitory Partial Pressures of CO_2, shown in the Case of White Mustard (*Brassica alba*); and also showing the Treatments by which Immediate Germination was induced.

Description of inhibited seed used.			Time seeds lay in germinating conditions in air after removal of inhibitory partial pressures of CO_2 and the sporadic germinations that occurred.						Percentage of seeds remaining ungerminated after 90 days in air in good conditions for germination.	Treatment adopted to induce germination.	Resulting germinations when re-set to germinate after treatment.
Time during which seeds remained with inhibiting partial pressures of CO_2.	Partial pressures causing inhibition in each case.	No. of seeds.	5 days.	10 days.	20 days.	30 days.	60 days.	90 days.			
3 weeks......	Per cent. 24–30	40	0	0	0				100	8 seeds, testas removed ...	All germinated at once.
										4 seeds, dried	,, ,,
										28 continued without treatment in germinating conditions	No germination indefinitely, but not dead.
7 days	14	20	1	1	1	1			95	Remainder dried 3 weeks	All germinated in 3 days.
	24	20	0	0	0	0			100	,, ,,	All germinated in 3 days except two, which germinated on removal of testa.
	32	20	1	1	2	4			80	,, ,,	All germinated in 3 days.
	40	20	3	3	5	7			65	,, ,,	All germinated in 3 days except one, which germinated on removal of testa.
	52	20	7	7	9	9			55	,, ,,	Do. do.
16 days	20	50	0	0	0	0	7	19	62	Remainder testas removed	All germinated at once.
	50	50	0	0	0	3	18	19	62	15 dried	Most germinated at once. Those that did not, germinated on removal of testa.
										15 continued without treatment	Sporadic germination continued.
										Remainder dried 35 days	All germinated at once.
	100	50	20	41					18		
46 days	24	10	0						100	Immersed in chloroform water 70 minutes	Sporadic germination continued. 32 days later seven had germinated.
46 days	24	10	0						100	Immersed in chloroform water 40 minutes	Sporadic germination continued. 32 days later two had germinated.

From the above tables of results it is clear that the case of *Brassica alba* seeds is peculiar in that the inhibition extends after the removal of the inhibiting CO_2 pressures from the atmospheres over the seeds. This after-inhibition may extend for months accompanied by a sporadic germination. The presence and condition of the testa seem to be the controlling factors in this after-inhibition. Germination is at once induced by the removal of the testa or usually by the complete drying of the seed.

(c) *Delayed Germination in Nature.*—From these experiments it is clear that a condition is produced in the seeds of white mustard after treatment with increased partial pressures of CO_2 which very closely parallels that of seeds showing delayed germination in natural conditions. This fact is brought out more clearly when the results obtained in the laboratory with *Brassica alba* seeds are compared with the results recorded by Nobbe and Hänlein (6) as occurring in nature. These authors give a large number of cases in which they observed indefinitely delayed germination in natural conditions accompanied by the sporadic sprouting of some of the seeds extending over long periods. A few examples may be given.

Table VI.—Extract from Nobbe and Hänlein's Tables of Seeds showing Delayed Germination in Natural Conditions.

Number and name of seeds.	Germinations after —— days.								
	5	7	8	16	145	351	519	874	Finally.
Capsella bursa-pastoris, 400 seeds	3	6	—	—	10	14	34	58	75
Thlaspi arvense	—	—	1	3	—	5	15	37	87

A similar result was obtained by these authors with a large number of species. The following may be mentioned:—*Chelidonium majus, Myosurus minimus, Plantago media, Potentilla argentea, Veronica beccabunga, Chenopodium album, Campanula rotundifolia, Campanula persicifolia.*

The similarity shown in the results obtained with the seeds of *Brassica alba* inhibited under the influence of CO_2 in artificial conditions to those demonstrated by Nobbe and Hänlein (6) as occurring in natural conditions is thus very marked.

Again, Crocker (5), working on a special case of delayed germination in the upper seed of the burr of Xanthium, which, in contradistinction from the lower seed of the burr, does not germinate in the first year after ripening but in the second, found that by removing the testa he could induce immediate germination at any time after ripening. The case of the inhibited seeds of *Brassica alba* offers an exact parallel to this case also.

(d) *Experiments Reproducing in Nature, with* CO_2 *Naturally Produced, the Results obtained in the Laboratory with* Brassica alba *Seeds.*—In drawing the foregoing parallels, a reflection which is suggested is that the inhibition of the *Brassica alba* seeds has been produced in the laboratory under conditions remote from those found in nature.

The following series of experiments were therefore directed to ascertain whether this objection is valid. The outcome of these experiments, it will be seen, is to indicate that the results of inhibition under the influence of CO_2 obtained in the laboratory with *Brassica alba* can be readily reproduced in the soil in conditions such as may occur widely in nature. The method of procedure was as follows:—Pits of various depths were dug in a garden soil consisting of sandy loam with very few stones. Short, fresh-cut grass was spread at the bottom in some cases. In others, green garden rubbish took the place of grass. The earth was then returned to the pits, and seeds, enclosed in small cotton-net bags, were inserted in it at various depths. The CO_2 content of the atmosphere of this soil at various depths was taken constantly during the experiments.

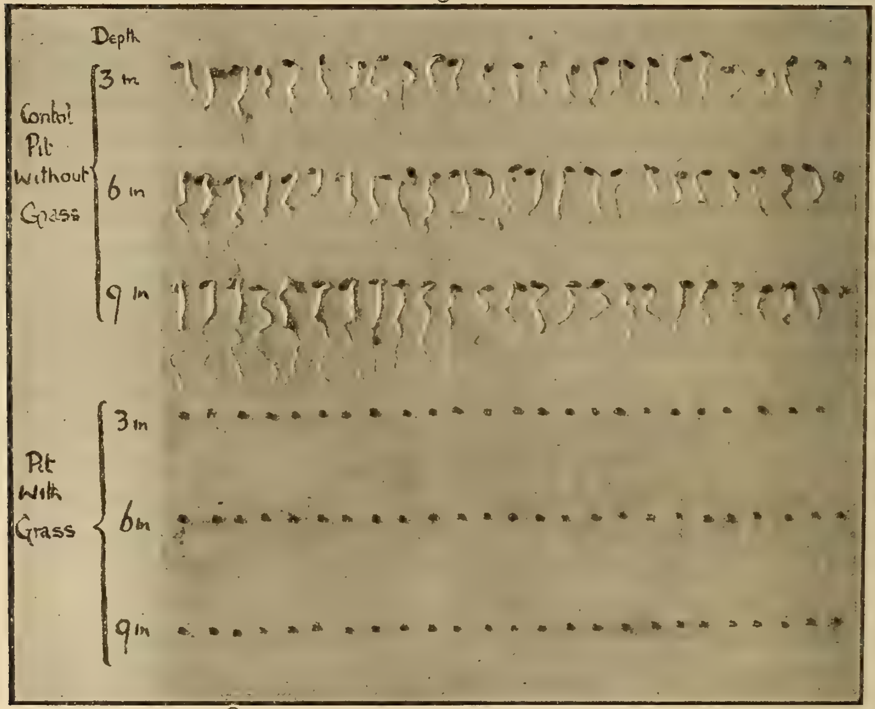
The following was a typical experiment:—On August 16, 1912, a pit 18 inches deep and 2 feet square was dug, a layer of packed green grass about 3 inches deep inserted, and the pit then filled up by the return of the earth removed. Seven days later, on August 23, three lots of 25 seeds each were buried at depths of 3, 6, and 9 inches in the earth in this pit over the grass. At the same time three control lots of seeds were placed at corresponding depths in a control pit close by, out of which the earth had been dug, and similarly returned seven days previously, but in which no grass had been placed.

The following are examples of the percentages of CO_2 found in samples of soil air taken during the experiment at depths of 6 and 12 inches in the pit containing grass:—

Per cent. Per cent.
August 23 at depth of 6 inches 12·4 CO_2; at depth of 12 inches 18·8 CO_2.
„ 29 „ „ 16·5 CO_2; „ „ 20·0 CO_2.

In the earth in the control pit, without grass, the CO_2 content of the soil continued steadily at about 1 per cent. at depth of 12 inches.

After seven days in the soil the seeds were removed and examined. None of those over the pit containing decaying grass had germinated at depths of 6 and 9 inches, while at a depth of 3 inches only 3 out of the 25 seeds had sprouted. All the seeds of the control lots at each depth in the pit without grass had germinated and sprouted vigorously. The results obtained are shown in the photograph and in the following table.



Results obtained with *Brassica alba* seeds in pit over Grass and in Control Pit without Grass after seven days.

Table VII.—Results obtained with *Brassica alba* Seeds, planted (1) in Soil over Decaying Green Grass, and (2) in Ordinary Soil.

Depths.	Germinations out of 25 seeds after 7 days.	
	Over decaying grass.	Control in ordinary soil.
inches.		
3	3 just sprouted	25 well grown.
6	0	25 ,,
9	0	25 ,,

Thus 72 out of the 75 *Brassica alba* seeds planted in soil over decaying grass were inhibited in conditions which may be supposed to occur sometimes in the soil (*e.g.*, in the ploughing in of green crops*). These seeds germinated sporadically afterwards, but systematic observations were not made in this first series of experiments as regards the after-behaviour of the inhibited seeds. A further series was, however, set in which the subsequent behaviour was noted. In this it was found that the results obtained with seeds inhibited in the soil closely conformed in all respects to the results obtained with those inhibited in laboratory conditions. In this experiment, which was conducted at a temperature of 5–7° C., and in which the seeds were left in the ground for 16 days, the CO_2 content in the soil over the buried grass rose from 10 per cent. on the 3rd day to 22 per cent. on the 16th. No germinations occurred with the *Brassica alba* seeds placed in the soil over the pit in which grass had been placed. All the seeds placed in the soil in the control pit without grass vigorously germinated within 10 days. When the inhibited seeds were removed to normal conditions of germination, 20 per cent. germinated sporadically within the first 10 days. The remainder were apparently living at the end of two months. None had been attacked by moulds. At this stage the testa was removed from a number of the seeds, with the result that germination was immediately induced, as in the laboratory experiments recorded above.

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* The case of heavily dunged land would also suggest itself. Boussingault and Lewy, in a large series of analyses of soil air, found 10 per cent. of CO_2 in manured soil 10 days

It would appear, therefore, that it is possible to reproduce in natural conditions, which may occur widely in the soil, the results obtained in the laboratory with inhibited *Brassica alba* seeds.

(e) *Action of the Testa. Bare Embryos inhibited by Carbon Dioxide.*—It is desirable now to return to the problem in its original form, in which it was indicated that germination may be due (1) to the action of a definite stimulus such as would be supplied by the access of oxygen under suitable conditions of moisture and temperature; or (2) to the removal of some inhibitory agent which has so far restrained the seed from entering upon the cycle of changes which begins with germination; or (3) to an inter-relation of both these causes.

In the experiments with carbon dioxide acting on the seed in germinating conditions so far related, it will be seen that certain partial pressures of CO_2 have the effect of retarding and inhibiting germination, the seed being capable of resuming growth without any apparent injury on the removal of the depressant. In the cases dealt with we seem to have two classes of results which must be separated. In the cases of all the seeds excepting

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after treatment. It appears from these results that caution is necessary in placing seed in the ground into which green crops have been ploughed or which has been recently heavily manured. In some of the experiments with pits described above the partial pressure of CO_2 in the soil atmosphere over buried grass was found to be as much as 8 per cent. seven months after the green grass had been buried.

Brassica alba it seems clear that the CO_2 has acted directly upon the tissues of the embryo. On the removal of the CO_2 the seeds readily germinate. In the case of *Brassica alba* the action of the carbon dioxide may have been the same, but on the removal of the CO_2 from the atmosphere the seeds do not germinate but continue dormant. A direct action of CO_2 on the testa, rendering it less permeable to the passage of gases, is suggested. Such a change in the testa produced by CO_2 would have two consequences: (1) a reduction in the amount of oxygen reaching the embryo, and (2) a relative rise in the CO_2 pressure in the embryo tissues. The possibility thus arises that lack of oxygen produced by a change in the permeability of the testa due to the action of CO_2 has been the cause of inhibition in all the experiments described.

The following experiment was therefore made with *Brassica alba* seeds from which the testas had been removed.

Table VIII.—Experiment indicating that Increased Pressures of CO_2 can act Directly in producing Inhibition on the Naked Embryo of *Brassica alba.*

Percentage CO_2 in air.	Time seeds lay without germinating in presence of high partial pressures of CO_2.	Numbers and condition of seeds set in presence of high partial pressures of CO_2.	Percentage of seed germinating on removal to air.	Percentage of germinated seeds showing injury to the radicle.
	days.			
60	2	10 without testas	100	0
80	7	10 ,, ,,	100	30

The above experiment appears to demonstrate that the inhibitory action of increased partial pressures of CO_2 may be direct upon the naked embryo of mustard seeds. The phenomenon of prolonged after-inhibition did not occur in these cases in the absence of the testa. Further experiments were made with peas and white mustard with similar results. A conclusion, therefore, which appears to be justified is that, while the inhibiting effect produced on the embryo is the result of the direct action of CO_2 thereon, in the case of *Brassica alba* an accompanying change in the testa plays an important part in sealing the seed under the influence of CO_2 in a special dormant phase of life.

Summary.

Experiments were conducted showing that the germination of seeds is retarded or inhibited by high partial pressures of CO_2 in the atmosphere.

This retardation or inhibition produced by CO_2 was shown to be unaccompanied by injury. The seeds used in these experiments fall into two classes. In the first class the seeds germinated at once after removal from the inhibitory CO_2 pressures (beans, cabbage, barley, peas, onions). In the second class the inhibition continued indefinitely after the removal of the inhibitory CO_2 pressures, and was terminated only by complete drying and re-wetting, or by the removal of the testa. In this class a lowering of the degree of permeability of the testa to gases by the action of CO_2 is indicated, a change which would have two results: (1) a reduction in the amount of oxygen reaching the embryo, and (2) a relative rise in the actual CO_2 pressure in the embryo tissues. The condition of prolonged inhibition after removal to air produced in *Brassica alba* is strikingly suggestive of the condition of seeds often met with in nature, the germination of which is delayed in spite of suitable conditions of temperature and water. The results obtained in the laboratory with *Brassica alba* seeds were reproduced in the soil in natural conditions by CO_2 arising from decaying vegetable matter. The high CO_2 content of the soil air in these experiments was found to continue for a considerable period. Attention was called to the importance of these facts in agriculture.

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The Functional Correlation between the Ovaries, Uterus, and Mammary Glands in the Rabbit, with Observations on the Œstrous Cycle.

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[Plates 17 and 18.]

Recent experimental work has resulted in proving that there is a definite functional correlation between the growth of the corpora lutea in the ovaries and the hypertrophy of the mammary glands (Ancel and Bouin and O'Donoghue). In the present paper experiments are described showing that this hypertrophy in rabbits that have never been pregnant may be so considerable as to lead to the production of milk, the secretion of which may be temporarily increased by the injection of pituitary extract. Further experiments are recorded showing that the uterus is not a necessary factor in the development of the mammary gland. The influence of experimentally produced corpora lutea upon the uterus is also described.

The Influence of the Ovaries upon the Mammary Glands.

It is well known that the mammary glands in man begin to undergo enlargement at the time of puberty in correlation with the increase in ovarian activity. Apart from this pubertal growth which is more or less permanent, there is known frequently to be a slight swelling of the glands at each menstrual period. A similar process takes place in the sow and probably in other mammals at the "heat" periods (Marshall). In the virgin rabbit we have noticed a growth of the mammary ducts in six cases prior to ovulation, but the cell proliferation, though quite definite, did not extend to the glandular tissue. Experiments were undertaken to determine if the growth could be increased by injecting fœtus extract, with a view to bringing further evidence to bear upon the hypothesis, put forward by Starling and Lane-Claypon, that the anabolic changes involved in mammary hypertrophy are dependent upon a fœtal hormone. The results, however, were negative in each case.

The following are the details of this series of observations. The extract was made by grinding the fresh fœtuses with sand and extracting with Ringer's fluid. The extract was then boiled and filtered. In the first three experiments described below 39 rabbit fœtuses were employed:—

(1) Fœtus extract was injected for 15 days into a virgin rabbit aged 5 months. The rabbit was then killed, when it was found that the ovaries contained a few follicles apparently ripe or nearly ripe, and the uterus a few glands. The mammary development was limited to ducts which were about 1 cm. long (fig. 1).

(2) In another virgin rabbit of the same age and treated identically the ovaries showed a degenerate follicle and a few follicles apparently ripe. The uterine glands were slightly developed, and the ducts of the mammary glands were fairly well developed, being about 1½ cm. long.

(3) Another virgin rabbit of the same age and treated identically gave similar results to the last (No. 2).

(4) A virgin rabbit, aged 5 months, was allowed to undergo a sterile copulation with a buck from which a portion of each vas deferens had been removed. It was killed 12 days after copulation. Contrary to expectation, no corpora lutea were found in the ovaries, but there was one large follicle. The uterus contained a few glands, and the ducts of the mammæ were about ½ cm. long.

(5) A virgin rabbit, 7 months old, was allowed to undergo a sterile coition with a vasectomised buck. It was killed 12 days afterwards. As in the last case (No. 4) no corpora lutea were found, but protruding follicles were present. The uterus had a few glands. The ducts of the mammary glands were well developed, being about 2½ cm. long.

(6) Another virgin, 7 months old, was allowed to undergo a sterile coition. It was killed 24 days later. There were no corpora lutea, but many protruding follicles, and the mammary ducts were about 2 cm. long.

It is thus seen that prior to ovulation the mammary development was limited to a slight cell proliferation in the ducts, and that the growth was not augmented by the injection of boiled fœtus extract. On the other hand, after ovulation (at least in the rabbit) definite mammary hypertrophy sets in, as will be described below.

Probably in the majority of mammals ovulation takes place spontaneously during œstrus. This is the case in the mare, the cow, the sow, the sheep (at least ordinarily), and the bitch. On the other hand, in the rabbit, the cat, and the ferret, ovulation, as a general rule, only occurs as a result of a stimulus set up by sexual intercourse. To which of these categories man belongs is still an open question.

It is generally believed that whereas the corpus luteum verum (or corpus luteum of pregnancy) and the so-called corpus luteum spurium (which is developed when pregnancy does not follow ovulation) are identical by origin, the structure formed after ovulation does not hypertrophy to the same extent as when pregnancy supervenes, but on the other hand undergoes retrogression after a few days. Ancel and Bouin, however, assert that in such animals as the rabbit the corpus luteum, when formed, undergoes the same amount of hypertrophy irrespective of the occurrence of gestation, and that since these animals do not normally ovulate excepting after coition the presence of corpora lutea is nearly always associated with the pregnant condition. Further, they put forward the view, for which a considerable body of evidence has been adduced, that in such animals as the rabbit the corpora lutea provide the exciting cause for the growth of the mammary glands during the first part of pregnancy.

In order to test this hypothesis they carried out experiments in which the Graafian follicles of rabbits were ruptured under such conditions that pregnancy could not supervene. The method usually adopted was to ligature the vasa deferentia of the male rabbits. This operation although inhibiting pregnancy, since spermatozoa cannot be ejaculated, does not prevent the occurrence of coition. Since coition without seminal ejaculation is generally sufficient to induce ovulation in the doe, corpora lutea could be formed just as though pregnancy had supervened. Ancel and Bouin found that the growth of the corpora lutea produced in this way was accompanied by a hypertrophy of the mammary glands which continued for about 15 days or until the corpora lutea began to undergo retrogressive changes. It was naturally concluded that the growth of the mammary glands was brought about by the activity of the corpora lutea. The further development of the mammary glands in pregnant rabbits is ascribed by Ancel and Bouin to the activity of a different gland, which is described as lying between the stroma and muscular layers of the uterus, and is designated the myometrial gland.

Frank and Unger have described a case of a virgin rabbit with corpora lutea in the ovaries and a breast development such as is usually characteristic of the end of the first third of pregnancy.

Furthermore, O'Donoghue has investigated the relation of artificially produced corpora lutea to the mammary glands. He took female rabbits in a condition of œstrus, and ruptured the Graafian follicles mechanically. In many cases corpora lutea were formed, and when this happened their presence was associated with a growth of the mammary glands. The amount of growth in 14 or 15 days is stated to have been about equivalent to that shown by the normal pregnant rabbit in 12 days. If, however, the artificial rupture of the follicles was not followed by the formation of corpora lutea the mammary glands did not show any hypertrophy. O'Donoghue had previously adduced evidence that the corpora lutea and mammary glands are functionally correlated in *Dasyurus*.

The following is an account of our experiments. In Experiments 7–17 the animals were all virgin prior to the occurrence of the recorded coition. The uterine changes are described separately below in dealing with the question as to the influence of the corpora lutea upon the uterus.

(7) A rabbit, 6 months old, was killed 3 days after a sterile copulation with a buck from which portions of each vas deferens had been removed. The ovaries contained corpora lutea. The ducts of the mammary glands were well developed, and there were slight traces of alveolar formation.

(8) A rabbit, 7 months old, was killed 5 days after a sterile copulation. There were corpora lutea of two ages present in the ovaries, and the mammary glands were well developed with the alveoli containing a secretion that appeared to be milk.

(9) A rabbit, 8 months old, in which the Fallopian tubes had been ligatured, was killed 9 days after a sterile copulation. The ovaries contained corpora lutea. The alveoli of the mammary glands were in process of formation.

(10) A rabbit, 8 months old, in which the Fallopian tubes had been ligatured, was killed 12 days after a sterile copulation. Sections through the ovaries showed that ovulation must have occurred in this case some considerable time (probably about 25 days) previously, since the corpora lutea were old and degenerate, and not recognisable on the surface of the ovaries. The mammary glands showed signs of involution, but milk was present in both the large and the small ducts. Milk could be expressed from the nipples before killing.

(11) A virgin rabbit, aged 7 months, was found to have ovulated spontaneously, this being very unusual in rabbits as above mentioned.* The ovaries contained corpora lutea, apparently about 14 days old. There were numerous alveoli found in the mammary glands (fig. 2).

(12) A rabbit, aged 15 months, from which portions of the Fallopian tubes had been removed, was killed 16 days after a sterile coition. The alveoli of the mammary glands were well developed.

(13) A rabbit, aged 8 months, was killed 24 days after a sterile coition with a vasectomised buck. Old corpora lutea were found in sections through one of the ovaries. The alveoli of the mammary glands were well developed and active, containing a granular milky secretion. Milk could be squeezed from the nipples. The milk was examined microscopically and

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* This rabbit was in a cage with another female. Doe rabbits in a state of œstrus when kept together have been observed to "jump" one another after the manner of cows when on heat, and it is possible that the stimulus set up in this way may be sufficient to induce ovulation.

stained with Sudan II, when fat globules were seen. The fluid when collected had the appearance of ordinary milk, and yielded a flocculent precipitate when treated with dilute acetic acid.

(14) A rabbit, 10 months old, was injected every day with boiled fœtus extract together with boiled placenta extract from the 11th to the 24th days, after a sterile copulation, the Fallopian tubes of the rabbit having been previously cut and portions removed. The rabbit was killed on the 27th day. Old corpora lutea were found in sections through the ovary. The alveoli of the mammary glands showed signs of atrophy, but the ducts and some of the alveoli contained milk. Before killing, a serous milky fluid was expressed from the nipples. The milk was tested as before, and found to contain some fat and albumen.

(15) A virgin rabbit, 11 months old, was rendered sterile by the Fallopian tubes being severed. It was then injected with boiled extract of uterus from the 11th to the 24th days, after a sterile coition. The rabbit copulated again on the 29th day, and was immediately afterwards killed. Old corpora lutea were found in the deeper parts of the ovary and several apparently ripe follicles on the surface. The mammary glands contained milk. Previously milk had been expressed from the nipples on the 19th, 21st, 27th, and 29th days after the sterile coition. The milk of this rabbit, collected in a test-tube, had the appearance of normal milk, and samples under the microscope were seen to contain globules of fat.

(16) A rabbit was injected with boiled uterus extract from the 11th to the 24th day after a sterile coition. Milk was expressed from the nipples on the 19th, 21st, 27th, and 29th days. The milk was collected and examined as in the previous case (No. 15). The rabbit copulated a second time with a vasectomised buck on the 29th day; 30 days later the rabbit copulated again and on the same day milk was expressed from the nipples; 28 days later milk was again expressed from the nipples. Next day the rabbit copulated again. Then on the same day 1 c.c. of pituitary extract was injected and the animal killed. The mammary glands were well developed, but showed signs of involution. They were full of milk. The ovaries contained old corpora lutea and ripe follicles.

(17) A rabbit was injected daily with boiled placenta and fœtus extract from the 11th to the 24th day after a sterile coition. Milk was expressed from the nipples on the 19th, 21st, 27th, and 29th days, and was collected and examined as in the preceding cases. On the 29th day (June 16) the rabbit again underwent a sterile copulation; 28 days later (July 14) although not pregnant, the rabbit plucked its fur from its breast and made a nest as if preparing for parturition. On the same day milk was expressed

from the nipples. Two days later (July 16) the animal again underwent a sterile coition. On the 17th day afterwards an attempt was made to express milk from the nipples but none could be obtained. On the 22nd day a little serous fluid was obtained, and on the 28th day a considerable quantity of fluid. The same day (August 13) the rabbit copulated a fourth time; 24 days later (September 6) no fluid could be expressed from the nipples, but two days later (September 8) a few drops of serous fluid were obtained. The rabbit copulated again (September 24) and is still alive.

(18) A multiparous rabbit underwent a sterile copulation with a vasectomised buck. On the 15th day after copulation no milk could be expressed from the nipples. On the 20th day milk could be obtained in considerable quantity. On the 22nd day the rabbit was again on heat and after undergoing copulation was killed. The mammary glands were found to be full of milk. Old corpora lutea and numerous degenerate follicles were found in sections through the ovary.

(19) This experiment was with a multiparous rabbit and the result was similar to that of the preceding experiment, there being no milk on the 15th day, but some milk on the 20th and 22nd days, on the latter of which the rabbit copulated again with a vasectomised buck (August 15). Twenty-two days later (September 6) a few drops of milk were obtained, and two days later quite a lot of milk was drawn off. The rabbit is still alive.

(20) This experiment was upon a multiparous rabbit which was exceptional in that no milk could be expressed from the nipples at any time during the period between two successive copulations with a vasectomised buck. After copulating a third time fluid could be expressed from the nipples on the 22nd day and on the 24th day.

(21) A multiparous rabbit was killed 17 days after a sterile copulation with a vasectomised buck. The mammary glands were found to be well developed but they contained no milk.

It is thus seen that in pseudo-pregnant rabbits (that is, in rabbits in which corpus luteum formation followed upon sterile coition) milk first made its appearance about the 19th day after copulation. At about this period the mammary hypertrophy appeared to have become complete and retrogressive changes set in, anabolism giving place to katabolism, at any rate to a considerable extent. These changes took place in the absence of any observed activity on the part of the myometrial gland, and it must be assumed that this gland is not an essential factor in mammary development. Moreover the immediate secretion of milk in considerable quantity followed by the characteristic changes in the tissue of the mammary glands could be induced by the injection of pituitary extract in the same kind of way as in normal lactation.

The interval between two œstrous periods (that is the interval occupied either partly or wholly by pseudo-pregnancy) was from 22 to 30 days, the period of gestation in the normal rabbit being 30 days.

Whether or not the corpus luteum plays any part in mammary growth or secretion in the latter stages of normal pregnancy is a point which has not been determined.

In normal pregnancy the development of the glands is undoubtedly greater than anything that occurs in pseudo-pregnancy, and it would seem probable that some further factor is concerned in bringing about this growth. This factor is possibly to be sought for either in the placenta, as suggested by Basch, or in the myometrial gland, as supposed by Ancel and Bouin. Nevertheless, it is clear that the presence of corpora lutea alone, apart from the existence of any subsidiary factor, suffices to stimulate gland growth to such a degree of completion as to result in the secretion of milk.

As mentioned already, Ancel and Bouin distinguish between the corpora lutea of pregnancy and the so-called "periodic corpora lutea" which only occur in animals that ovulate spontaneously. The artificially produced corpora lutea in the rabbit are regarded as belonging to the former kind. Moreover in those animals (like the rabbit) which only ovulate after coition the interstitial cells are supposed to take the place of the periodic corpora lutea. It may be doubted whether the distinction made between the two kinds of corpora lutea by Ancel and Bouin should be insisted upon. In the first place the corpora lutea are all formed in precisely the same way from the discharged follicles, while according to Biedl the ovarian interstitial cells in rodents arise from connective tissue which grows inwards so as to fill up the cavities of degenerate follicles. Such cells are designated by Seitz "theca lutein cells" since they arise in the theca interna of the follicles, and subsequently develop into cells resembling those of corpora lutea. Miss Lane-Claypon, however, states that the ovarian interstitial cells are derived, like the follicular epithelial cells, from the germinal epithelium.*

Furthermore, from the account given by Hill and O'Donoghue it would seem that the corpora lutea in *Dasyurus* always undergo the same degree of development irrespectively of the occurrence of pregnancy. They describe an animal as being seen to clean out its pouch for the reception of young, although it had not become pregnant, thus showing that in *Dasyurus* the cyclical changes of the sexual organs, which are apparently consequent upon ovarian changes, may even extend to the instincts associated with parturition and the nursing of the young, although pregnancy had not taken place.

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* I have noted the presence of interstitial cells in the ovary of the rabbit prior to the maturation of any follicles.—J. H.

A case of a rabbit which prepared a bed for a litter and secreted milk at the termination of the pseudo-pregnant period has been recorded above. Cases have also been reported by various observers of similar instincts in bitches, which have been described as making preparations for parturition and secreting milk nine weeks after coition although they had failed to become pregnant. Thus Heape records instances of bitches which had been "lined" but had "missed" having pups, yet had secreted milk at the time when they were due to whelp, in sufficient quantity to admit of their rearing litters belonging to other bitches. Cases have also been recorded by Noel Paton. Moreover, several such cases of bitches which did not conceive but yet have afterwards yielded milk have been recently reported to the authors.

It is suggested that in these animals the building up of the mammary glands and the resulting secretion of milk may have taken place in response to a stimulus arising in corpora lutea which developed after œstrus and possibly persisted for an abnormal length of time. If this explanation is correct it is clear that no essential distinction can be drawn between the corpora lutea of pregnancy and the periodic corpora lutea in regard to their functional relation to the mammary glands.

Our observations lend no support to the theories of Starling and Lane-Claypon, Foà, Biedl and Koenigstein, who have supposed that the mammary glands are built up under the influence of a hormone arising in the fœtus, neither are they confirmatory of the view put forward by Halban, who regards the placenta as a factor in mammary growth. Our experimental results are, at first sight, somewhat difficult to reconcile with the facts observed by Ott and Scott, and Schäfer and Mackenzie, who found that corpus luteum extract (like that of pituitary) when injected into the circulation has an immediate galactogogue action. It must be borne in mind, however, that the sudden injection of considerable quantities of corpus luteum extract into the circulation is not a process which occurs in nature, and consequently we might expect its effect upon the mammary tissue to be different from that of small quantities of the problematical hormone when continuously secreted over a long period.

The Effect of Hysterectomy without Ovariotomy.

Experiments were also undertaken to ascertain whether or not the uterus is an essential factor in mammary growth. As already mentioned, Ancel and Bouin have expressed the opinion that in the later stages of pregnancy the myometrial gland of the uterus is an exciting cause in mammary development. It occurred to us that it was possible that the uterus might also be an essential factor in bringing about mammary development in the earlier stages of pregnancy, and that the corpora lutea might be unable to exert their influence upon the mammæ excepting through the mediation of the uterus. The changes which the uterus undergoes (to be described below) as a result of the formation of the corpora lutea lent a certain amount of evidence in favour of this view. It had, however, been shown that the removal of the uterus in young rabbits has no effect upon the subsequent growth of the ovaries, for animals so operated upon after becoming mature are capable of copulation, ovulation, and the formation of corpora lutea just as though they had not undergone hysterectomy; but the effects (if any) of the removal upon breast development were not recorded (Carmichael and Marshall).

The following is an account of our experiments:—

(22) The uterus was removed from a virgin rabbit when 10 months old. Subsequently the animal copulated and was killed 25 days after copulation. No remains were found of the uterus or Fallopian tubes, and one ovary was missing, presumably having become absorbed as a result of vascular interference at the time of the hysterectomy operation. The other was normal and contained nine corpora lutea. The alveoli of the mammary glands showed signs of atrophy, but it was clear that they had undergone a considerable growth previously. Both alveoli and ducts contained a secretion.

(23) The uterus was removed from a virgin rabbit when 3 months old. After it had reached maturity it was allowed to copulate several times, and killed 12 days after the last copulation. One ovary contained four corpora lutea, the other having undergone atrophy. No remains of uterus or Fallopian tubes could be found. The mammary glands showed a great development of alveoli but no milk was present (fig. 3).

(24) The uterus was removed from a virgin rabbit when 3 months old. After it had reached maturity it was allowed to copulate several times, and was killed 9 days after the last copulation. The left ovary contained several corpora lutea. Small pieces of the Fallopian tube were found attached to it. The right ovary had undergone partial atrophy presumably as a result of vascular interference, and there was a small piece of the right Fallopian tube with a cyst. The mammary glands were well developed, the alveoli being filled with a secretion.

(25) The uterus was removed from a virgin rabbit when 3 months old. The rabbit subsequently copulated. A little serous fluid could be squeezed from the nipples on the 22nd and 27th days after copulation. The rabbit copulated again on the 28th day (August 14). A few drops of fluid were expressed 25 days afterwards (September 8), when it copulated again and was immediately killed. The mammary glands were well developed. The

alveoli and ducts were full of a milky secretion. Both ovaries contained corpora lutea. There were no remains of tubes or uterus.

(26) The uterus was removed from a virgin rabbit when 3 months old. On the 27th day after copulation (which took place when maturity was reached) fluid could be squeezed from the nipples. On the 28th day the rabbit was killed, when it was found that the alveoli of the mammary glands were well developed. The ovaries contained corpora lutea. There was a small piece of one Fallopian tube left.

(27) The uterus was removed from a virgin rabbit when 3 months old. It reached maturity, copulated, as in the preceding cases, and was killed 17 days later. No remains of uterus or tubes could be found. The ovaries contained eight (three and five) corpora lutea. The mammary glands were well developed, the ducts and alveoli being filled with a secretion.

These experiments show that mammary development occurring in rabbits as a result of the formation of experimentally produced corpora lutea takes place independently of any uterine influence. Thus the uterus is not a factor in mammary growth any more than in ovarian growth. The experiments show further that the presence of one ovary, with its contained corpora lutea, is sufficient to bring about the mammary hypertrophy.

The Influence of the Corpora Lutea upon the Uterus.

It has been concluded by Fraenkel and others that the corpus luteum is an essential factor in the fixation of the fertilised ovum to the uterine wall and in the nourishment of the embryo during the first stages of pregnancy. This conclusion is based on the results of ovariotomy during early pregnancy and on a large number of control experiments. Whether or not the evidence is sufficient to justify the theory being stated in precisely this form, it would seem clear that the development of the corpus luteum is functionally connected with the contemporaneous hypertrophy of the uterine wall during the first stages of gestation, since the raised nutrition of the uterus is dependent upon the presence of the corpus luteum (Marshall and Jolly). Ancel and Bouin state that in the case of the rabbit the non-pregnant uterus undergoes hypertrophic changes when corpora lutea are developed. This has been called in question by Dubreuil and Regaud, but Niskoubina's observations are confirmatory of those of Ancel and Bouin.

The following is an account of our observations upon the changes undergone by the non-pregnant uterus after ovulation consequent upon sterile coition (excepting in the case of Experiment 11 where the rabbit had ovulated spontaneously). The condition of the ovaries and mammary glands has been already described. The numbers of the experiments provide a means of identifying the individual rabbits previously referred to. The respective ages of the rabbits, which prior to the occurrence of the recorded coition were all virgins, have also been given above.

(7) In a rabbit killed 3 days after sterile coition the uterine glands were just commencing to undergo active growth.

(8) In a rabbit of 5 days the glands were considerably developed and the muscular walls had undergone some thickening.

(9) In a rabbit of 9 days the process had been carried further (fig. 5).

(10) In a rabbit of 12 days the uterine glands were more numerous and smaller than those of No. 9. They were also more closely packed, and the uterus showed congestion. The muscular walls were very thick. It is to be noted again that the ovaries contained very old corpora lutea (see above).

(11) This rabbit had ovulated and corpora lutea were present, apparently about 14 days old. The uterus showed a great development of glands which were elongated and formed a spongy-looking mass at the base of the folds. The muscular coat was thickened.

(12) In a rabbit killed 16 days after sterile coition the uterine glands were enlarged and spongy-looking. The capillaries in the stroma between the glands were distended. The muscular layers were very thick.

(13) In a rabbit of 24 days the uterine glands were smaller than those of No. 12, but still very active. The folds of the mucosa contained a large amount of extravasated blood, showing that the congestion had resulted in a breaking down of the blood-vessels. The muscular coat was moderately thick (fig. 6).

The changes outlined above presented an essential similiarity to those described by Hill and O'Donoghue for the pseudo-pregnant marsupial cat. There is a strikingly close likeness between the appearances which we have just described (as shown in sections through the rabbit's uterus during the successive stages) and the figures published in Hill and O'Donoghue's paper on *Dasyurus*. In view of this great similarity there can hardly be reason to doubt that the changes which take place in the rabbit's uterus after sterile coition are physiologically homologous with the changes which occur in the uterus of *Dasyurus* during the period of pseudo-pregnancy. As will be shown subsequently the recognition of this fact, which has not hitherto been pointed out, materially affects the views entertained by the above-mentioned authors regarding the nature of the homology between the œstrous cycle of the marsupial and that of the Eutherian mammal.

Lastly, the hypertrophic changes which take place in the uterus during pseudo-pregnancy are clearly comparable to those which occur in true pregnancy in association with the development of the embryo, whose presence necessitates the maintenance of a raised nutrition on the part of the organ which protects it and through which it derives nourishment. That the corpora lutea are a factor in preserving this raised nutrition seems to have been established beyond question.

The Œstrous Cycle.

According to Heape a period of five or six months (*i.e.* spring and summer) is the usual duration of the sexual season in the domesticated rabbit. Heape says further: "No doubt if they are kept warm, carefully fed, and their breeding carefully regulated throughout the spring and summer, they may exhibit œstrus also in winter, but it must be recollected that here we are treating of œstrus independently of pregnancy, which is a very different matter."

Our experience has been different from that of Heape, for many of our rabbits, kept in hutches in an outhouse and without any artificial heating, have bred in the winter months, though not with the same frequency as in spring and summer. The following is a record :—

	Percentage breeding.
Of 12 rabbits which copulated about Dec. 14, 5 had young	41·7
,, 24 ,, ,, Mar. 22, 14 ,, ...	58·3
,, 21 ,, ,, May 18, 17 ,, ...	81·0
,, 8 ,, ,, June 14, 8 ,, ...	100·0

Half of these rabbits had been treated with Yohimbine, administered by the mouth for several days before copulation, but the drug, although in other cases it caused a pronounced congestion of the uterus, did not increase the breeding powers or affect the fecundity, as compared with the other rabbits which were kept as controls.

Prof. Punnett, who has kindly supplied us with further information concerning the recurrence of œstrus in rabbits, finds that when kept in a moderate temperature, produced when necessary by artificial heating, not only is there very little, if any, restriction of the sexual season to a particular time of the year, but that copulation in the winter is followed by pregnancy. The following is a record of the œstrous periods (so far as observed) and times of litters for one of Prof. Punnett's rabbits from October, 1910, to May, 1912:—

Put to male.	First notes made on litter.
Sept. 27, 1910	Oct. 29, 1910
Jan. 26, 1911	Feb. 27, 1911
Mar. 29, 1911	Apr. 30, 1911
June 2, 1911	July 4, 1911
July 15, 1911	Aug. 19, 1911
Sept. 2, 1911	Oct. 6, 1911
Oct. 25, 1911	Nov. 27, 1911
Jan. 12, 1912	Feb. 15, 1912
Feb. 15, 1912	Mar. 17, 1912
Apr. 11, 1912	May 13, 1912
May 17, 1912	June 19, 1912

Heape states that 10–15 days is the average duration of the diœstrous cycle, but that some individuals exhibit heat at intervals of three weeks.

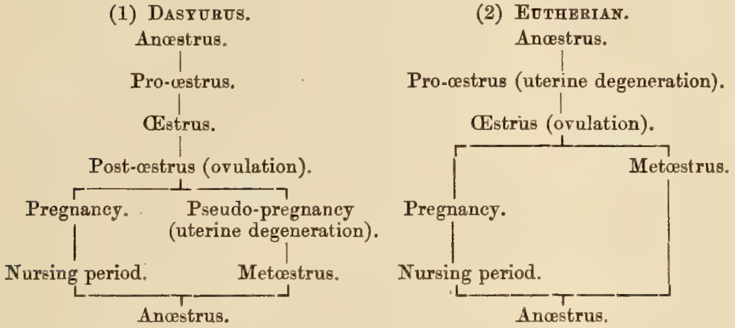
The pro-œstrum is stated to last from one to four days, and œstrus for about a day or longer. During the pro-œstrum the vulva tends to become swollen and purple in colour, and this appearance may continue during œstrus. There is no external bleeding, and it is difficult or impossible to state when the pro-œstrum ends and œstrus begins. It would seem that the two periods are much abbreviated, as in the case of the sheep and many other animals in which the uterine changes characterising the heat periods are slight, as compared with those of the dog or the monkey.

The uterus may show undoubted congestion at the heat period, but we have never observed any breaking down of vessels or extravasation of blood in the non-pregnant rabbit's uterus, excepting near the end of the pseudo-pregnant period. It is possible that these (or some of these) cases represented the commencement of a pro-œstrous period. Apart altogether from these instances congestion presenting a close similarity to that observed in the case of the pro-œstrous sheep was found to occur in the rabbit's uterus at the time of heat. Pigment formation has not been noticed. Its absence from the uterus of the rabbit suggests that in this animal blood extravasation does not ordinarily take place in the pro-œstrous or œstrous periods. The glands do not show very much evidence of activity during the heat period, and their degree of development is very much less than that shown in the earlier stages of the pseudo-pregnant period.

Theoretical.

Many of the observations described above have an important bearing upon certain statements made by Hill and O'Donoghue in a recent paper on the œstrous cycle in the marsupial cat, *Dasyurus viverrinus*. According to these authors ovulation in *Dasyurus* occurs at an interval of some days after œstrus, there being a definite post-œstrous period terminating in ovulation.

Further, it is stated that the degenerative changes in the uterine mucosa of the marsupial instead of preceding ovulation, as they do in the dog, take place after ovulation during a period which, in the non-pregnant animal, is designated the period of pseudo-pregnancy. The differences in the reproductive cycles are shown in the following scheme drawn up by Hill and O'Donoghue:—*


(1) Dasyurus.
Anœstrus.
Pro-œstrus.
Œstrus.
Post-œstrus (ovulation).
Pregnancy.
Pseudo-pregnancy (uterine degeneration).
Nursing period.
Metœstrus.
Anœstrus.
(2) Eutherian.
Anœstrus.
Pro-œstrus (uterine degeneration).
Œstrus (ovulation).
Metœstrus.
Pregnancy.
Nursing period.
Anœstrus.

Hill and O'Donoghue express the opinion that the degenerative changes seen in *Dasyurus* during the pseudo-pregnant period are equivalent to those which take place in the Eutherian during the pro-œstrum. They suggest that the shortening of the cycle in the Eutherian may have induced an increased growth of the mucosa during the pro-œstrum, and that this in time may have conditioned the earlier recurrence of the degenerative and regenerative changes, with the result that these have been shifted forward so as to occur prior to ovulation instead of after it. On the other hand, Hill and O'Donoghue appear to hold the view, which seems to us scarcely consistent with the suggestion just quoted, that menstruation in man is a degeneration of the uterine mucous membrane, due to its being unable to fulfil its purpose owing to the absence of a fertilised ovum. They state, further, that their observations "afford no support to the view that 'menstruation is identical with heat' nor for the view that 'menstruation in the Primates is the physiological homologue of the pro-œstrum in the lower Mammalia.'" Thus they appear to regard the condition existing in the Primates as directly comparable to that occurring in *Dasyurus*, and different from the condition found in the dog.

Our own observations on the rabbit indicate that the changes in the non-pregnant uterine mucosa which take place concurrently with the development of the corpora lutea are essentially similar to those described for *Dasyurus* in the period of pseudo-pregnancy. The close likeness between the sections of the rabbit's uterus and the figures given in Hill and O'Donoghue's paper has been commented on above. Moreover, the processes which take place in the ovaries and mammary glands are also clearly of an identical nature in the two animals. We suggest, therefore, that the uterine changes which go on in the pseudo-pregnant uterus in the marsupial are not comparable to the pro-œstrous changes of the Eutherian, as Hill and O'Donoghue suppose, but are identical with those in the pseudo-pregnant rabbit's uterus, both being dependent upon the formation of corpora lutea in the ovaries. It is possible, however, that the uterine congestion occurring near the close of the pseudo-pregnant period is of the nature of a pro-œstrous congestion, since pseudo-pregnancy (like true pregnancy) would probably in some cases have been followed by another œstrous period, had the animals been permitted to live.

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* In the scheme drawn up Hill and O'Donoghue "Diœstrus" is unaccountably inserted for the non-pregnant Eutherian between "Pro-œstrus" and "Metœstrus." In the scheme as given above, this is omitted, since the diœstrous period, when it occurs in polyœstrous animals, supervenes r metœstrum and not before.

It has been shown by Hill and O'Donoghue that in the marsupial cat there is only one sort of corpus luteum, the duration of which is presumably always the same. In the rabbit, also, there is only one kind of corpus luteum occurring in correlation with either pregnancy or a condition comparable to pseudo-pregnancy. The existence of only one kind of corpus luteum (which lasted for an identical period, irrespectively of whether or not ovulation was succeeded by pregnancy) was no doubt the condition common to all primitive mammals, and it seems probable that the shortening of the duration of the "periodic corpus luteum"* was associated with the development of the polyœstrous habit from a state of monœstrum. For it is known that ovulation cannot ordinarily occur in the presence of fully developed corpora lutea, which, if they persist, cause follicular atrophy and inhibit the development of ripe ova. Consequently it would be disadvantageous for such animals to have periodic corpora lutea persisting for as long a period as corpora lutea associated with pregnancy.

In monœstrous animals, such as the dog, the persistence of the corpus luteum over a period equivalent to pregnancy would not be detrimental to fecundity, while we have shown above that there is evidence (derived from numerous cases where bitches have been known to secrete milk nine weeks after œstrus) that even in the dog such a persistence may occur. Moreover, the great variability which different individual dogs experience in the recurrence of œstrus is suggestive of a variation in the period over which the corpus luteum persists. It may be that in monœstrous animals the primitive condition occurring in *Dasyurus*, in which there is one sort of corpus luteum only, continues to exist or is reverted to in certain individuals.

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* Or corpus luteum spurium.

Summary and Conclusions.

(1) The development of the corpus luteum of pregnancy, or of pseudo-pregnancy, in the rabbit is functionally correlated with the hypertrophy of the mammary glands, as already shown by Ancel and Bouin, and by O'Donoghue.

(2) This hypertrophy is followed on about the 19th day after coition, in pseudo-pregnant rabbits, by a definite secretion of milk, the quantity of which may be temporarily augmented by the injection of pituitary extract, just as in normal lactation.

(3) The mammary hypertrophy can take place in rabbits from which the uterus has been removed while still immature, thus showing that the uterus is not an essential factor in the development of the mammary glands.

(4) The development of the corpora lutea of pseudo-pregnancy is further correlated with uterine hypertrophy and hyperæmia followed by extravasation of blood.

(5) These uterine changes are clearly comparable to those which occur in true pregnancy, and afford a confirmation of the view that the corpora lutea are a necessary factor in causing and maintaining the raised nutrition of the uterus during the first part of the period of gestation.

(6) The changes which take place in the rabbit's uterus during pseudo-pregnancy are homologous with those which occur in the uterus of the marsupial cat during pseudo-pregnancy, and these latter are not pro-œstrous in character (at any rate, in the earlier stages) as Hill and O'Donoghue suppose.

(7) The domesticated rabbit is capable of breeding throughout the whole year, but less frequently in winter than in spring or summer. If corpora lutea of pseudo-pregnancy are produced, the recurrence of œstrus is postponed until these are in an advanced stage of retrogression.

(8) The shortening of the duration of the so-called corpus luteum spurium of many mammals has probably been brought about in correlation with the acquirement of the polyœstrous condition.

The injections referred to in this paper were done by J. Hammond; the operations by F. H. A. Marshall. The work was carried out at the Field Laboratories, Cambridge, in connection with the School of Agriculture. The expenses have been defrayed by a grant made by the Board of Agriculture and Fisheries out of money allotted to it, for purposes of research, by the Development Commissioners.

[*Postscript, March* 6, 1914.—In describing the results of hysterectomy we omitted to mention that Foges found that the uterus was not a factor in the pubertal growth of the mammary glands.

Aschner and Grigoriu in a recent paper describe the effects of injecting placental extract into virgin guinea-pigs. Development of the glands followed, and this was succeeded by milk secretion. In the guinea-pig ovulation may take place spontaneously, so that it is probable that there was some gland development before the injections were made. Ovarian or placental extract was found to cause hyperæmia and other changes in the uterus.

Fellner has lately described marked changes in the uterus and mammary glands of the rabbit after injecting extracts of corpus luteum and placenta. The organs affected are said to have undergone a considerable hypertrophy, but milk production could not be induced.

Steinach has recorded experiments on guinea-pigs in which the ovaries of females were transplanted into males and produced breast development.

Doncaster in a very recent paper on sterility in cats records a case of what may be regarded as milk production following upon pseudo-pregnancy. Longley had previously observed that the cat, like the rabbit, normally ovulates only after coition. One of Mr. Doncaster's cats after copulating with a tortoise-shell male failed to become pregnant. It occurred, however, to one of the present writers that since copulation had taken place it was probable that corpora lutea had been formed though unaccompanied by pregnancy. It seemed possible, therefore, in the light of our experiences with rabbits that the cat in question might secrete milk. This was found to be the case four weeks after the last copulation, and Doncaster records that the secretion continued for about two weeks subsequently.]

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DESCRIPTION OF PLATES.

Plate 17.

Fig. 1.—Microphotograph of mammary tissue of virgin rabbit (Experiment 1, p. 423). The mammary development is limited to a few ducts.

Fig. 2.—Microphotograph of mammary glands of virgin rabbit which had ovulated spontaneously about 14 days previously (Experiment 11, p. 425). The glands contained numerous alveoli.

Fig. 3.—Microphotograph of mammary glands of rabbit from which the uterus had been removed while still a virgin. It was killed 12 days after copulation (Experiment 23, p. 430). The glands showed a great development of alveoli.

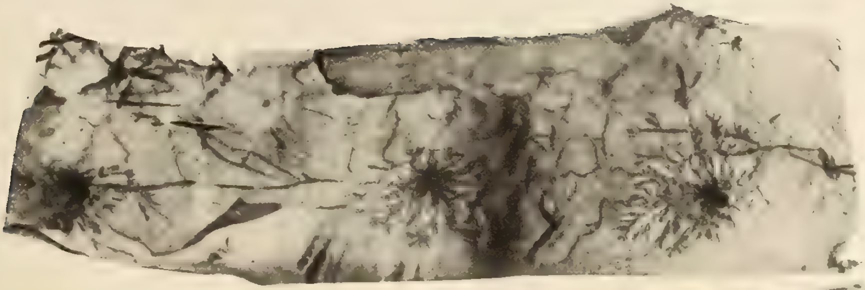
Plate 18.

Fig. 4.—Section through portion of mammary gland of rabbit 24 days after sterile coition (Experiment 13, p. 425). The alveoli contain milk. × 78.

Fig. 5.—Section through uterine mucosa of rabbit nine days after sterile coition (Experiment 9, p. 432). The glands are very greatly developed. × 35.

Fig. 6.—Section through uterine mucosa of rabbit 24 days after sterile coition (Experiment 13, p. 432). A large quantity of extravasated blood is present. The glands are still somewhat enlarged. × 35.

Figs. 4–6 were drawn by Mr. Edwin Wilson, of Cambridge.



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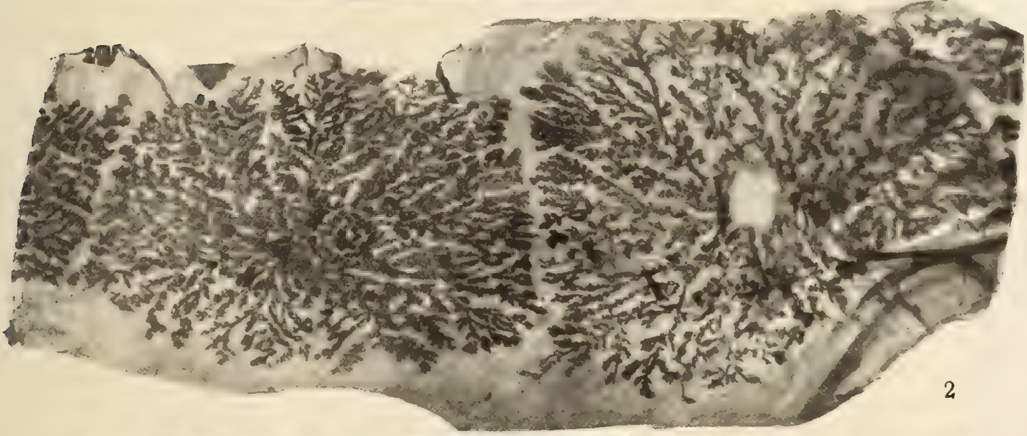

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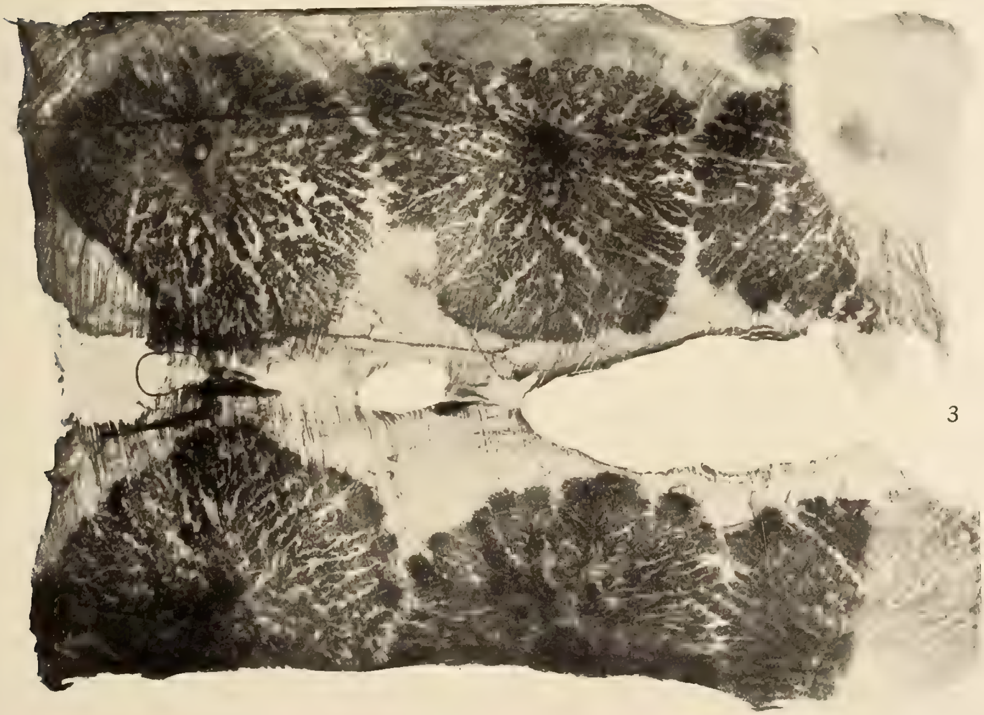

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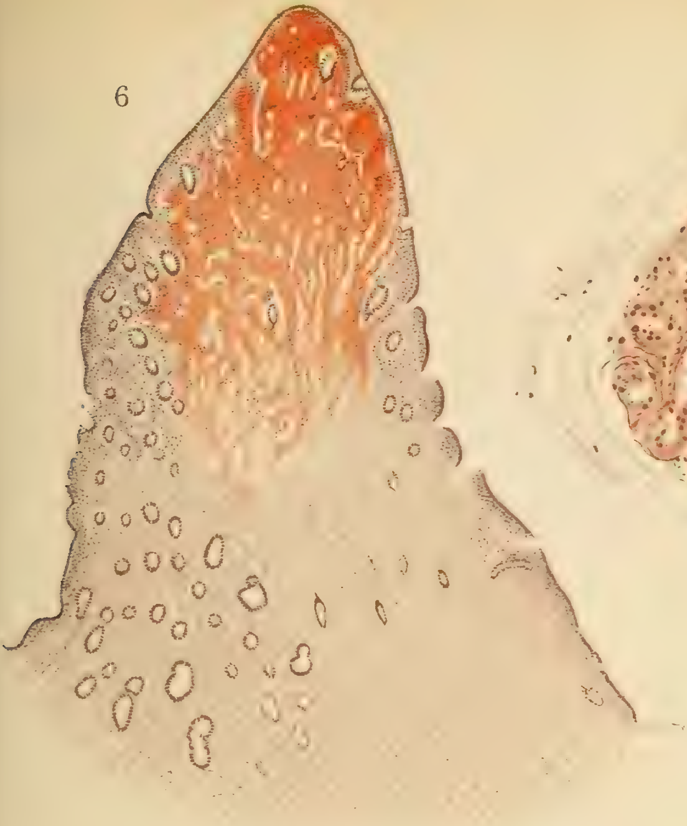

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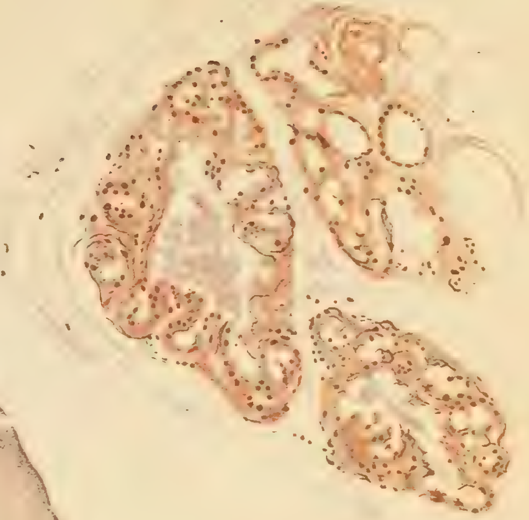

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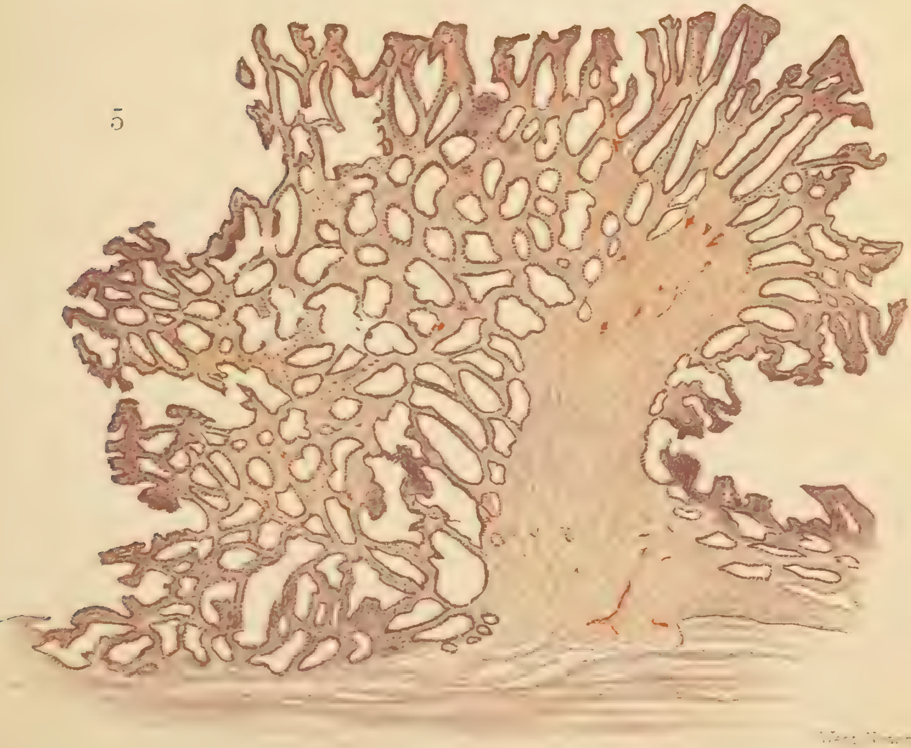

Oxidation of Thiosulphate by Certain Bacteria in Pure Culture.

By William T. Lockett.

(Communicated by Prof. P. F. Frankland, F.R.S. Received February 14,—Read March 26, 1914.)

In the course of investigations on the oxidation of thiosulphate on bacterial sewage filters,* it was found that partially oxidised filtrates, still containing appreciable quantities of thiosulphate, were slowly but completely oxidised by simple aëration in the presence of living organisms, practically no oxidation taking place in the control experiments with corresponding solutions rendered sterile by steaming.

Further investigations were undertaken with a view to the isolation of the organism or organisms capable of bringing about this oxidation.

Accordingly, gelatine and agar plates were made from active filtrates from time to time and in general about 100 organisms per cubic centimetre were noted, which were mainly of the non-liquefying and chromogenic types. Subcultures in peptone water and peptone broth of several of the predominating types were made and after a few days' incubation added to solutions of thiosulphate, which were then aërated under sterile conditions. Many experiments were carried out in this manner without success. Variations were introduced with regard to the age of the cultures and the nature of the culture media, without effect, practically no oxidation of the thiosulphate solutions taking place after several weeks' aëration.

Subsequently it was observed that a bacteriological slide made of a loopful of an active filtrate showed proportionately a greater number of organisms per cubic centimetre than was indicated by the gelatine and agar plates of the same solutions. Further, the microscopic appearance of these—consisting mainly of one particular type—was very different from that of the organisms previously subcultured.

All attempts to grow the particular and characteristic organism on the usual media, *e.g.* nutrient gelatine and nutrient agar, failed. Minor investigations indicated that the organism was most active in neutral solutions containing only small quantities of organic matter, whilst ammonium sulphate was a decided stimulant.

Ultimately it was found on plating out very small quantities (*e.g.* 0·001 c.c.) of an active filtrate on a solid gelatine medium made without bouillon, but containing ammonium sulphate (0·1 per cent.) and sodium thiosulphate (0·4 per cent.), that a great number of slow growing, circular, non-liquefying, bluish-white colonies were obtained.

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* 'Journ. Soc. Chem. Ind.,' June 16, 1913, vol. 32, No. 11, p. 579.

Plates made with such a medium showed that active filtrates contained 100 to 1000 times more organisms per cubic centimetre than was shown on gelatine and agar. In addition the microscopic appearance of the organism was apparently identical with those previously noted in the slides of the filtrates.

Streak cultures of the organism made on media of the same composition as the above produced clearly defined, thin, bluish-white growths after 10 to 15 days' incubation at 20° C.

Experiments were then made to ascertain how far this particular organism was able to bring about the oxidation of thiosulphate. At first difficulties arose with regard to the finding of a suitable liquid medium for the growth of the organism; eventually good growths were obtained by the use of a medium of the following composition:—

1·0 grm. sodium thiosulphate, 0·5 grm. ammonium sulphate, 0·5 grm. potassium biphosphate, 0·025 grm. sodium chloride, 0·01 grm. magnesium sulphate, 2·0 grm. Rochelle salt, dissolved in 1000 c.c. distilled water.

To this solution it was found necessary to add sufficient acid (N H_2SO_4) to reduce the alkalinity to methyl orange by approximately one-half, thereby presumably liberating free tartaric acid. Before and after sterilisation clear solutions were obtained of this mixture, which were alkaline to methyl orange.

A suitable solid medium for the growth of the organism is also obtained by the addition of gelatine (10 per cent.) to this solution.

In testing the oxidising power of the organism the procedure generally adopted was as follows:—A pure streak culture was taken, and a small quantity of the growth, attached to the end of a sterile platinum needle, was introduced into 10–12 c.c. of the above sterile solution contained in a test-tube, the usual bacteriological precautions being observed.

After a few days' incubation at 20° C. a slight white, stringy growth was observed in the inoculated solutions. Later, after 14–21 days a distinct turbidity was apparent, and the solutions on examination at this period were found to be free from thiosulphate. Complete oxidation had taken place with the formation of acid sulphate, the final solution being slightly acid to methyl orange.

Uninoculated solutions showed no change after several weeks' incubation.

A large number of experiments have been made on these lines with complete success. Solutions inoculated directly from colonies found on ammonium sulphate gelatine plates were similarly oxidised, and other experiments have been conducted which confirm the above results.

The following is a typical example of the chemical results obtained :—

Results in parts per 100,000.

	Oxygen absorbed in three minutes from acid permanganate.	Reaction with mercurous nitrate.
Inoculated solution after 21 days' incubation at 20° C.	1·00	White ppt.
Solution of control experiment after 21 days' incubation at 20° C.	28·80*	Black ppt.

* Equivalent to 83·8 parts $Na_2S_2O_3$ per 100,000.

That the thiosulphate is bacterially oxidised to sulphate and that the change is not a simple decomposition due to the formation of acid by the organism seems evident from the fact that (1) there is no deposition of free sulphur, (2) the final solutions do not absorb appreciable amounts of oxygen from acid permanganate, this excludes the presence of thionic acids.

The following are comparative results obtained with three solutions, to one of which had been added before incubation 1 c.c. of normal sulphuric acid, thereby making the solution decidedly acid to methyl orange :—

Results in parts per 100,000.

	Oxygen absorbed in three minutes from acid permanganate.	Reaction with mercurous nitrate.	Remarks.
1. Inoculated solution after 21 days' incubation at 20° C.	1·80	White ppt.	Slight turbidity.
2. Solution of control after 21 days' incubation at 20° C.	21·00*	Black ppt.	Clear solution.
3. Solution made decidedly acid to methyl orange prior to 21 days' incubation at 20° C.	14·60	Yellow ppt. (thionic acids).	Deposit of sulphur.

* Equivalent to 61 parts $Na_2S_2O_3$ per 100,000.

The organism is apparently able to live in slightly acid solutions, although prolonged contact with free acid appears seriously to impair its activity and growth.

Further experiments are in progress relating to the morphology and classification of the organism, which appears to be one hitherto unknown, and to its effect on other sulphur compounds, *e.g.* tetrathionate.

My thanks are due to Prof. P. F. Frankland, Dr. G. J. Fowler, and Edward Ardern, M.Sc., for the interest which they have taken in this work and to the Rivers Committee of the Manchester Corporation for permission to publish the results of this investigation carried out in the laboratory of their sewage works at Davyhulme.

The Production of Anthocyanins and Anthocyanidins.

By ARTHUR ERNEST EVEREST, M.Sc., Lecturer in Chemistry, University College, Reading.

(Communicated by Prof. F. Keeble, F.R.S. Received February 16,—Read March 26, 1914.)

The idea that the anthocyan pigments are closely related to the flavone and flavonol glucosides is by no means new. Attempts to solve the problem of their relationship have come chiefly from botanists, and, as a result of their researches, a number of hypotheses have sprung up around which quite considerable controversy has been centred.

Miss Wheldale* puts forward the suggestion that anthocyan pigments are the oxidation products of colourless or faintly coloured chromogens; and that these chromogens are products of hydrolysis of glucosides present in the tissues of the plant (probably glucosides of flavone or flavonol derivatives). The hydrolysis of the glucoside she considers as essential to the production of the anthocyan pigment. She represents the changes taking place by means of the following equations:—

$$\text{Glucoside} + \text{water} \rightleftharpoons \text{chromogen} + \text{sugar.}$$

Then— Oxidation of chromogen → anthocyan pigment.

If this hypothesis be accepted, then either the anthocyan so produced will remain a non-glucoside, *i.e.*, it will be an anthocyanidin, or in the presence of sugars the anthocyanidin first formed must unite with sugar to form an anthocyanin (glucoside). Her more recent suggestion that in flavone glucosides all the hydroxyl groups are substituted by sugar molecules, hence partial hydrolysis could produce glucoside anthocyans,† has apparently no foundation upon experimental evidence, most of the flavone and flavonol glucosides containing one or two sugar residues only.

Now, in view of the fact that it has recently been shown that in no case could any trace of anthocyanidin be found in any of the plants examined,* there remains but one way of explaining their absence if Miss Wheldale's hypothesis is to be retained; namely, by assuming that the rate of formation of anthocyanin (glucoside) from anthocyanidin and sugar is greater than that of the production of anthocyanidin from chromogen by oxidation, and that these reactions take place under similar conditions.

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* 'Camb. Phil. Soc. Proc.,' vol. 15, p. 137 (1909); 'Journ. Genetics,' vol. 1, p. 133 (1911).

† 'Biochem. Journ.,' vol. 7, p. 87 (1913).

If this were correct one would expect that by taking the yellow glucoside, hydrolysing, then reducing without removal of sugars, the anthocyanidin produced would combine with the sugar present to form an anthocyanin. This is not the case. Evidence all tends to show that Miss Wheldale's view can no longer be accepted as explaining the available facts.

The reaction found so useful in determining the presence or absence of glucosidal or non-glucosidal anthocyan, and already described by Willstätter and Everest (*loc. cit.*), yields a very ready means of distinction between these two classes of compounds, and has led to important results in the present investigation. It depends upon the facts that anthocyanidins (non-glucosides) are taken quantitatively from aqueous acid solutions, preferably sulphuric acid, by shaking with amyl alcohol, whereas anthocyanins (glucosides) remain quantitatively in the aqueous acid when similarly treated; and, further, that the non-glucoside in amyl alcohol, shaken with sodium acetate solution, remains quantitatively in the amyl alcohol, but on shaking with sodium carbonate solution it is quantitatively carried down into the aqueous layer.

The author has been able to show the production of anthocyanins from yellow glucosides, and that in the cases where hydrolysed solutions were taken only anthocyanidins were produced. That no intermediate formation of anthocyanidins occurred when anthocyanins were obtained was shown by carrying out the formation under amyl alcohol. Where glucoside yellow pigments were used the anthocyanin appeared as usual, but no anthocyanidin passed into the amyl alcohol; when a hydrolysed solution was similarly treated the amyl alcohol rapidly took up all the anthocyanidin as it was produced.

This makes it necessary to abandon the assumption suggested above as the only explanation available if Miss Wheldale's hypothesis is to remain.

A number of papers have been published upon this subject by Keeble, Armstrong, and Jones,† and they conclude that the anthocyan pigments are produced in a manner similar to that propounded by Miss Wheldale, but they part company with that author in regard to the process necessary subsequent to hydrolysis of the glucosides, for they maintain that the oxidation must be preceded by reduction of the non-glucoside flavone or flavonol derivative.

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* Willstätter and Everest, 'Annalen,' vol. 401, p. 189 (1913).

† 'Roy. Soc. Proc.,' B, vol. 85, p. 214 (1912); B, vol. 86, pp. 308 and 318 (1913); B, vol. 87, p. 113 (1913); and Keeble and Armstrong, 'Journ. Genetics,' vol. 2, part 3, p. 277 (1913).

The result of work already published on the pigment of the cornflower,* and consideration of the work of Keeble, Armstrong, and Jones, have led the author to the conclusions (1) that if the anthocyans are produced from the yellow glucosides, then it must be by some interaction in which the glucosides and not the hydrolysed glucosides take part; and (2) that all evidence obtained in dealing with the above-mentioned pigments tends to show that the anthocyan pigments would prove to be, not oxidation, but reduction products of the yellow glucosides.

That luteolin and morin give red pigments on reduction in acid alcoholic solution by means of sodium amalgam has been known for many years.† Quite recently Watson‡ has obtained a red pigment from quercetin by the same means, and, lastly, since the present work was completed, the author's attention has been drawn to a paper by R. Combes,§ who describes the production by the same means from the yellow pigment of the green leaves of *Ampelopsis hederacea* of a pigment which he shows to be identical with the red pigment (anthocyan) which he obtained from the red leaves of the same plant. He does not, however, give definite information whether his pigments are anthocyanins or anthocyanidins. The author is able to confirm the production of anthocyan pigments by reduction of flavone or flavonol derivatives, and to explain the observations of Keeble, Armstrong, and Jones. Being one of the most commonly occurring of the flavonol class, and readily obtained, quercitrin (Kahlbaum) was taken for the starting point.

Quercitrin by reduction with zinc (fine granulated) and dilute acids (2N HCl) or by electrolytic reduction, even cold, gave only anthocyanidin.

The pigment production took place equally well when the aqueous acid was covered with a layer of ligroin, so precluding all possibility of oxidation by the air following reduction. As, however, no anthocyanin could be obtained from quercitrin (this is a monoglucoside from bark, not a flower pigment, at least some flowers are known to contain a diglucoside of quercitrin, *e.g.*, viola contains viola quercitrin)—a result which at first appeared to support Miss Wheldale's hydrolysis hypothesis—the author decided to test the pigments obtained by direct extraction of various flowers, in particular with a view to producing anthocyanins from the yellow glucosides present in them.

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* Willstätter and Everest, *loc. cit.*

† *Cf.* Rupe, 'Die Chemie der natürlichen Farbstoffe,' vol. 1, pp. 77 and 85.

‡ 'Chem. Soc. Proc.,' 1913, p. 349.

§ 'Compt. Rend.,' vol. 157, p. 1002 (1913); 'Chem. Zentr.,' 1914, p. 158.

Having already shown that oxidation after reduction was not necessary for the production of anthocyan pigments—and this was confirmed in every case where reduction under ligroin was carried out—particular attention was given to proving that anthocyanins could be produced directly from yellow glucosides without intermediate formation of anthocyanidins, and in this the author was successful.

The yellow wallflower, yellow daffodil, white narcissus, yellow or white tulip, white primula (*obconica*), yellow crocus, yellow jasmin, yellow primrose (the presence of yellow pigments in the white flowers was shown by action of dilute ammonia, when, the plant acids being neutralised, the yellow colour appears), and even lemon peel, all yielded by reduction alone red pigments, and, indeed, pigments which upon investigation proved to be in every case an anthocyanin, no trace of anthocyanidin being produced when the reductions were carried out in the cold. No oxidation after reduction was necessary for the production of the anthocyanin pigment, provided that in one or two instances care was taken not to carry the reduction too far.

Reduction was carried out by zinc (fine granulated) in *ca.* 2N aqueous acids, and also by electrolysis in 2N sulphuric acid, using lead electrodes (lead has been found to yield salts with anthocyanins, which, however, are decomposed by acids; lead salts have no harmful effect upon anthocyanins).

At first some difficulty was experienced in explaining the observations of Keeble, Armstrong, and Jones* that in the case of yellow wallflower, yellow daffodil, yellow crocus, cream polyanthus, and Chinese primrose oxidation was necessary after reduction in order to obtain a red pigment. A ready explanation was, however, forthcoming when the case of the yellow tulip was examined, for here, when reduction was rapid, there appeared but a transient pink, passing rapidly to a colourless solution, which, however, on addition of hydrogen peroxide immediately developed a red colour. Slow reduction, however, by zinc (very small quantities) and HCl or, much better, slow electrical reduction gave readily the red pigment, and this proved to be as in the other cases an anthocyanin. The red solution on stronger reduction passed to a colourless one, from which the anthocyanin was again produced by the addition of hydrogen peroxide.

It has been found that in each case excessive reduction produced to a greater or less extent the above result, and this clearly explains the results of Keeble, Armstrong, and Jones (*loc. cit.*).

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* 'Roy. Soc. Proc.,' B, vol. 87, p. 113 (1913).

On a previous occasion attempts to reduce cyanin (the pigment of the cornflower*) to a colourless compound which could be re-oxidised to the pigment had failed. Powdered zinc and acetic acid were used, hot—the pigment was decolorised, but the red colour was not reproduced on addition of hydrogen peroxide. Despite this fact, the author, for comparison, treated a small quantity of cyanin chloride in 2N hydrochloric acid with much finely granulated zinc, so that vigorous evolution of hydrogen ensued. The reaction was carried out in the cold, and, as in the cases mentioned above, decolorisation rapidly set in, but on decanting the decolorised solution and adding hydrogen peroxide the colour reappeared. The glucoside was not hydrolysed by this process.

In every case, also with cyanin chloride, when treated with hydrogen peroxide in the cold, the red acid solution of the anthocyanin passed to a yellow, then became colourless. It would thus seem that the balance of reducing powers present in an anthocyan-containing flower must be very finely adjusted, for it appears necessary that the reducing body present should be powerful enough to reduce as far as the anthocyanin stage, but not powerful enough to take the pigment further to the colourless condition.

It has been placed beyond doubt that the change from yellow to red may be accomplished by reduction alone, thus confirming the results of Combes, and, still further, that the change from glucoside flavone or flavonol to anthocyanin (glucoside) takes place quite readily without hydrolysis, and that all hypotheses which require a hydrolysis of the glucoside before formation of red pigments can, in the light of the evidence of Willstätter and Everest, that the anthocyanidins do not exist in plants, and the further evidence now brought forward, that flavone or flavonol glucosides readily yield anthocyanins without intermediate formation of anthocyanidins, be discarded as unnecessary.

Whether all the yellow glucosides of the flavone and flavonol series are capable of producing corresponding anthocyanins remains to be proved by future work. The author failed to observe such formation in the case of *Primula sinensis* (Giant white), mimosa, and white hyacinth. (Whites tested with ammonia gave yellow.)

Whether the red pigments described above should be considered as mere hydroflavone derivatives as I, or as some such anhydro-compound of them, II, remains to be proved, but the author considers that the evidence at present available favours some such form as II, where the change has caused the production of a quinonoid structure, as follows :—

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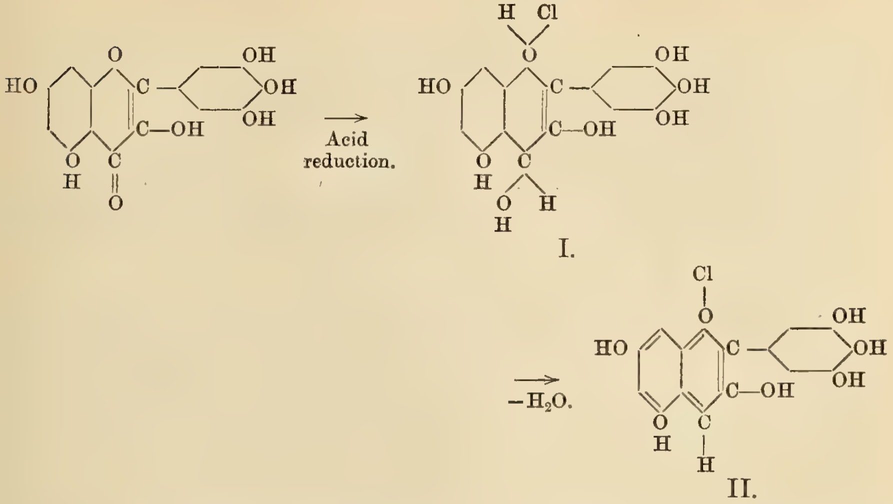
* Willstätter and Everest, *loc. cit.*



In this connection an examination of the properties of cyanin chloride and cyanidin chloride* is of interest. Cyanidin chloride when heated for a short time in dilute alcohol to *ca.* 80° becomes decolorised—the decolorised substance has properties resembling those of a yellow flavonol pigment, soluble in ether, colourless in acid solution, extracted from it by ether; yellow in alkaline solution, and alkalis withdraw it from its solution in ether. The decolorised cyanidin chloride, however, on boiling with acids, returns to the red form. It is possible that these changes may be represented by the change from I to II above being a reversible reaction. The fact that a decolorised solution of cyanidin chloride on concentration regains its colour also harmonises with the above.

Quite similar properties are observed in the case of cyanin chloride, save that heat is not required for decolorisation, nor for return of the red pigment on acidification. Extraction of the decolorised solution with ether was not tried by Willstätter and Everest, but alkalis on the decolorised solution gave a yellow coloration. Most probably the general character of the groups in the molecule would have their effect on the readiness with which this change took place, and hence this decolorisation. Such a change might perhaps explain the observation of Keeble, Armstrong, and Jones (*loc. cit.*), that in the case of polyanthus mere boiling with acid was sufficient to produce the red pigment.

As he learns that recent developments in the work of Prof. Willstätter and his collaborators have caused them to commence a series of investigations dealing with the relation between the yellow pigments and the anthocyans the author proposes to discontinue these investigations for the present.

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* Willstätter and Everest, *loc. cit.*

Experimental.

Quercitrin.—Reduction carried out in 2N HCl by zinc (fine granulated).

(1) Hot, yielded rapidly a red solution.

(2) Cold, gave red coloration but very slowly.

(3) Cold, alcoholic HCl and Na Hg: rapidly gave red pigment.

(4) Cold, electrolysis in 2N sulphuric acid, lead electrodes: very slow production of red.

(5) The best method, however, of obtaining the red pigment from quercitrin is by the action of magnesium (ribbon or turnings) on a solution of the substance in a mixture of 5 vols. absolute alcohol and 1 vol. concentrated hydrochloric acid. Not only does this go very readily, but the acidity of the solution—so essential in working with these compounds—is preserved as the magnesium practically ceases to react before the solution becomes neutral. This method was of no value when working with crude plant extracts, as alcoholic extracts contained so much extraneous matter that the results were masked.

In every case pigment, when shaken with amyl alcohol, went quantitatively into the alcohol, solution red with tinge of violet; shaken with sodium acetate solution, pigment remained quantitatively in alcohol, turned violet; shaken with sodium carbonate solution, pigment descended quantitatively into aqueous layer with green colour; prepared by method (5) and purified from remaining quercitrin, solution gave blue solution in sodium carbonate. The red pigment was not extracted from aqueous acid by any other organic solvent.

Yellow Wallflower.—Petals from a few flowers crushed in mortar with fine sand and cold 2N HCl, then filtered, gave a faintly yellow extract which with ammonia became deep yellow. To one portion of acid extract a small quantity of zinc was added, whilst a second portion was kept for comparison, to show that no red developed without the reduction. In a few minutes the portion containing zinc became pink and the colour rapidly deepened to red. Blank portion remained unchanged. The pigment produced by reduction remained quantitatively in the aqueous layer when shaken with amyl alcohol, but if the layer was separated, then boiled to hydrolyse the pigment, and then again shaken with amyl alcohol, the red pigment then went quantitatively into the alcoholic layer with production of a red solution. This reacted in every way as an anthocyanidin. Electrolysis also produced the anthocyanin and only that, no trace of anthocyanidin was produced by reduction in the cold.

Reduction in hot solution produced anthocyanidin and no anthocyanin.

Reduction under ligroin produced the same results as without protection from air.

Primula obconica (white).—Petals gave clear yellow on treatment with ammonia, no pink with acids. Extract made as above, almost colourless. Reduction with zinc in cold 2N HCl gave good red pigment. The reduction went equally well under ligroin, and in both cases the pigment produced was quantitatively anthocyanin, and could be hydrolysed quantitatively into anthocyanidin.

Primula sinensis (Giant white).—Petals gave clear yellow with ammonia, no pink with acids. All attempts to obtain a red pigment failed.

Tulip (yellow).—Extract prepared as above in 2N HCl. With much zinc a faint passing pink colour appears, then solution becomes decolorised, hydrogen peroxide added to the decanted solution causes appearance of red colour. Exposure to air has same effect. The red produced is an anthocyanin readily hydrolysable to an anthocyanidin.

When acid extract was treated with small quantities of zinc the pink colour soon appeared and deepened. If not taken too far hydrogen peroxide caused no change.

Electrolysis of cold extract in 2N sulphuric acid, lead electrodes, readily gave the red pigment which, as in the preceding cases, proved to be entirely anthocyanin.

Tulip (white).—Petals with ammonia gave clear yellow. Exactly similar results were obtained as for the above yellow tulip. Both in the case of yellow and white tulips the reduction went on equally well under ligroin.

When the extract from the white tulip was boiled to hydrolyse the glucoside contained, then cooled and reduced in the cold, a red pigment was readily obtained, but it was entirely an anthocyanidin.

Daffodil (yellow).—Extract as before, zinc in 2N HCl gave red pigment easily. Electrolysis in 2N sulphuric acid gave same result. In both cases cold reduction gave only an anthocyanin. Reduction went on to red pigment equally well under ligroin.

Narcissus (small white).—Petals with ammonia gave clear yellow. Reduction with zinc in 2N HCl gave only anthocyanin.

Mimosa.—All attempts to get red pigment failed.

Hyacinth (white).—Petals gave yellow with ammonia, but all attempts to obtain red pigment failed.

Crocus (yellow).—Extract as above gave, by zinc in 2N HCl, or by electrolysis in 2N sulphuric acid, red pigment quite readily, in both cases cold reduction yielded only anthocyanin.

Jasmin (yellow).—Gave anthocyanin only, more readily by means of zinc and 2N HCl than by electrolysis.

Primrose (yellow).—Easily produced anthocyanin by either method, even in fairly warm HCl with zinc only anthocyanin was produced. The glucoside produced in this case seemed to be more stable to hydrolysis than in the majority of cases.

Lemon Peel.—Extract in HCl, in presence of the peel, reduced with zinc gave only anthocyanin.

Variations in the Growth of Adult Mammalian Tissue in Autogenous and Homogenous Plasma.[*]

By Albert J. Walton, M.S., F.R.C.S., B.Sc.

(Communicated by Prof. W. Bulloch, F.R.S. Received February 18,—
Read March 26, 1914.)

(From the Bacteriological Laboratory of the London Hospital.)

[Plates 19 and 20.]

In 1910 Carrel commenced his researches on the growth of tissues outside the body. In 1907 Harrison had succeeded in growing the embryonic tissues of the frog, using coagulable lymph as a medium. In 1910 Harrison and Burrows improved this method and successfully cultivated the tissues of mammalian embryos. Carrel has so modified the technique that the method is now applicable to the study of the growth of all mammalian tissues. He used as a medium the plasma of the animal either in its natural state or modified by the addition of various substances. Since then, he and his collaborators have published a large number of papers, and by their work it has been fully established that tissues of animals, whether embryonic or adult, grow well *in vitro*; that by changing the medium and so removing the catabolic substances life can be greatly prolonged—tissues have been kept alive and growing for periods considerably longer than a year; and that the growth of the tissues can be greatly modified by the addition of various substances to, or otherwise altering the composition of, the plasmatic medium.

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[*] Throughout this paper the term "autogenous" is used to indicate plasma obtained from the same animal as the tissue, "homogenous" to indicate that obtained from another animal of the same species.

In previous communications I have described fully the characters of the growth of adult mammalian tissue in primary and in sub-cultures. Having determined the nature of this growth, it became possible for me to investigate the variation, if any, of the growth in autogenous and homogenous plasma. The results of this investigation are detailed in this paper.

The tissues of adult rabbits were used and the technique of Carrel was carefully adhered to. Four hundred and thirty-three cultures were made, the testicle, thyroid, and kidney being the tissues mainly used. A few experiments were made with tissues of the spleen but owing to the amount of emigration of cells, apart from true growth, which occurs with this tissue it was found difficult to make accurate comparative experiments. These experiments were therefore discontinued.

The details of the experiments on the testicular tissue and the results will be fully described. The other tissues will be considered more briefly as the experiments on them were carried out on the same lines.

Testicle.

One hundred and twenty-two cultures were made with testicular tissue. It was found that the plasma of the rabbit kept in ice would not continue fluid for longer than about an hour. After this time it coagulated and became useless. To overcome this difficulty the plasma was placed in a sterile tube which was corked and sealed with paraffin, and the tube was immediately placed in a mixture of salt and ice contained in a thermos flask. By changing the mixture of salt and ice every two or three days the plasma could be kept frozen hard for an indefinite time. When required for use it was removed from the mixture and at room temperature soon became fluid.

Experiment 1.—Plasma was removed from Rabbit A six days previous to the cultural experiment and kept frozen. At the time of the cultural experiment blood was collected from the carotid artery of Rabbit B and the plasma separated. Cultures of testicle of Rabbit B were made in the usual way in both plasmata. Twelve cultures were made in the homogenous plasma and twelve in the autogenous. Of those in the homogenous, good growth occurred in all, whilst of those in the autogenous plasma only eight grew and growth was less extensive in these.

Experiment 2.—Cultures of testicle were made in homogenous plasma which had been kept frozen for three days and in fresh autogenous plasma. In both the same testicular tissue was planted and the cultures were made at the same time and under identical conditions. The cultures in homogenous plasma again gave 100 per cent. of positive results whilst those in autogenous plasma gave only 75 per cent. positive. The growth was again more extensive in the homogenous plasma.

Although the above experiments seemed to show that growth was better in homogenous than in autogenous plasma, it was possible that in both cases the autogenous plasma happened to be what one may describe as a bad variety. To solve this question a third experiment was performed three days later than the second experiment. The same two plasmata which were used in the second experiment had been preserved frozen and were used again. They were now both homogenous to the culture tissue and were respectively six and three days old.

Experiment 3.—The two frozen plasmata described above were used, one of which was six days and the other three days old. Cultures of testicle were made in these and in fresh autogenous plasma under similar conditions. The results were somewhat similar to those of the last two experiments. Growth was much more extensive in the homogenous than in the autogenous plasma, thus at the end of three days there was but slight growth of the tissue in the autogenous plasma and marked growth in the three-day-old homogenous plasma, 100 per cent. of the pieces growing. In the case of the six-day-old homogenous plasma it was seen that the growth was more extensive than in the autogenous plasma but less than in the three-day-old homogenous plasma, only 70 per cent. of the pieces growing. Sub-cultures were made from each set and it was again seen that after a period of three days growth was much more extensive in the homogenous than in the autogenous plasma. In the homogenous plasma mitotic figures were very abundant and very well marked.

This experiment showed that growth was not better owing to the accidental choice of good homogenous plasmata, for the plasma which in Experiment 2 was autogenous and gave but poor growth when used in Experiment 3, where it was homogenous, gave a good growth in 100 per cent. of the trials. Another very interesting fact became apparent. The homogenous plasma used in Experiment 2 when three days old was successful in 100 per cent. of the trials, but when used in Experiment 3, that is when six days old, gave less growth, and even this appeared in only 70 per cent. of the tissues. These results are shown tabulated on p. 455.

The facts suggested that the variations in growth might be dependent upon the length of time that the plasma had been kept frozen, and further experiments were therefore carried out to elucidate this point. No more sub-cultures were made, however, for it was evident that if the same plasma were used it would not be of the same age and therefore further variants would be introduced.

	Animal A.	Animal B.
Plasma 1	Homog., 3 days old. Good, 100 per cent.	Homog., 6 days old. Medium, 70 per cent.
Plasma 2	Autog., fresh. Slight, 75 per cent.	Homog., 3 days old. Good, 100 per cent.
Plasma 3	—	Autog., fresh. Fair, 60 per cent.

Experiment 4.—Plasmata were removed from two other animals and kept frozen, one eleven and the other eight days before the cultural experiment. Just before this experiment was commenced blood was removed from the lateral ear vein of another animal and the plasma separated. Cultures were then made under identical conditions in the four plasmata, viz.: homogenous eleven days old, homogenous eight days old, homogenous fresh, and autogenous fresh. In the first two groups every piece of tissue died and there was no evidence of growth. The tissues in fresh plasma grew in the usual way and to an equal extent. The growth in the autogenous plasma was perhaps a little more extensive than that in the homogenous plasma (Plate 20, figs. 5 and 6).

These experiments showed that testicle grew better in homogenous plasma that had been kept frozen for three days, but not at all in plasma that had been frozen for more than six to eight days. The question as to whether growth was better in autogenous or homogenous plasma was still undecided. The following experiment was therefore devised to settle this point.

Experiment 5.—Two rabbits were taken. Blood was removed by puncture from the lateral ear vein of each, ten and three days before the cultural experiment, the plasma being separated and frozen. At the time of the experiment blood was removed from the carotid artery of each and the testicle taken out. Thus there were obtained from each animal three plasmata, one which had been frozen for ten days, one for three days, and one fresh, that is six in all. The testicle of each animal was cultivated in all the plasmata, making twelve separate groups. The cultures were fixed at the end of 48 hours and stained so that the early growth-characters might be seen, these being considered more capable of comparison than the later stages when the growth was well advanced.

In the case of the testicle taken from animal A there was no trace of growth in the ten-day-old plasma, whether taken from animal A or animal B. With the three-day-old plasma that from animal A, autogenous, showed well marked growth, but that from animal B, homogenous, showed very slight growth and marked vacuolation of the plasma. With the fresh plasma there was a fair amount of growth in both series, but whereas that in the autogenous plasma (fig. 4) was considerably less than that in the three-day-old plasma, that in the homogenous was greater than that in the three-day-old homogenous plasma and rather less than that in the fresh autogenous. In the case of the testicle taken from animal B, there was again no trace of growth in the plasma from either animal which had been frozen for ten days (fig. 3), but in the three-day-old plasma there was marked growth in the plasma from animal A, which in this case was homogenous (fig. 2), and little or no growth in the plasma taken from animal B, which in this case was autogenous. With the fresh plasma there was growth in both series, but that in plasma A, homogenous, was more marked (fig. 1) than that in plasma B but was much less than that in the three-day-old homogenous plasma. These results are shown in the following table:—

	Animal A.		Animal B.	
	Plasma A. Autogenous.	Plasma B. Homogenous.	Plasma A. Homogenous.	Plasma B. Autogenous.
10 days	0	0	0	0
3 days	Very good	Slight	Very good	Slight
Fresh	Good	Fair	Good	Fair

The above experiments showed that, as regards the testicle, growth was not dependent upon any variation in the nature of the cells, for growth was equally good in the series whichever testicle was taken, but it varied directly with the plasmatic medium which was used. The variations in the plasma were not specific to either autogenous or homogenous tissues, for in the experiments given above tissues from both animals grew in the one plasma whether it was autogenous or homogenous, whereas in the other plasma they grew badly in either case. Some plasmata give good growth and others but little, but at present there is not sufficient evidence to show upon what these differences depend.

The fact that growth was always better in plasma that had been frozen for a certain time, whereas, if kept frozen for a longer period, growth entirely ceased, seemed to show that each plasma contains two substances, one of which inhibits growth and the other which stimulates it. By exposure to freezing for two or three days we may suppose the inhibitory substance is destroyed so that growth is increased. After a longer period, about eight days, the stimulating substance is also destroyed and hence there is no growth. Under normal conditions the stimulating substance is in excess of the inhibitory substance, therefore a certain amount of growth takes place in fresh plasma. In plasmata which are not "good" only a small amount of growth takes place when the plasma has been frozen for three days. This is not so easy to understand; it may be that the stimulating substance is present in a less marked degree, and is therefore all destroyed at an earlier date, so that after the plasma has been frozen for three days there will be little or none present, hence growth will be slight or absent. It was noticed, however, in the cases above where growth was slight that coagulation of the plasma had been incomplete; in some cases, indeed, the plasma had remained quite liquid, so that there was risk of the tissue washing off the slide. It is possible therefore that failure to grow under such circumstances was due to mechanical factors, the plasma failing to form a scaffolding for the growing cells. It is of interest to note that the plasma which failed to coagulate was not serum, for there was no clot present when the frozen material was thawed.

Thyroid.

Of this tissue 167 cultures were made, the experiments being carried out on similar lines to those described for the testicle, but a larger number of cultures were made, so that the plasmata were compared at shorter intervals of time.

Experiment 6.—Homogenous plasma was removed one day previous to the cultural experiment and frozen. Autogenous plasma was removed from the animal at the time of the experiment and cultures of thyroid tissue made in each plasma under identical conditions. Growth was more marked in the homogenous plasma and a greater number of cultures were positive in this.

Experiment 7.—Thyroid tissue was cultivated in eight days' old homogenous plasma and in fresh autogenous plasma. There was no growth in the homogenous plasma, whereas in the fresh autogenous plasma 42 per cent. of the cultures grew and the amount of growth was well marked.

Thus, as in the case of the testicle, growth is better in plasma that has been preserved for one day, but entirely ceases in plasma which has been frozen for eight days.

In the next series the same plasmata were used for several experiments, as in the case of the testicle, so that any given plasma which was autogenous in one experiment became homogenous in the next, and had been kept frozen for periods of time which increased for each successive experiment.

Experiment 8.—Thyroid tissue was cultivated in fresh autogenous plasma and in the plasma used in Experiment 7, which was now five days old.

The homogenous plasma gave 100 per cent. positive results and growth was very well marked in it. Only 13 per cent. grew in the autogenous plasma and growth in it was slight.

Experiment 9.—Thyroid tissue was cultivated—

(1) In plasma taken from animal 7, now nine days old.

(2) In the plasma taken from animal 8, now four days old.

(3) In fresh autogenous plasma.

In the nine-day plasma 60 per cent. of the cultures grew, the growth being fairly extensive.

In the four-day plasma 67 per cent. grew, and the growth was very well marked.

In the fresh autogenous plasma 44 per cent. of the tissues grew, and growth in these was less extensive than in the other groups.

Experiment 10.—Thyroid tissue was cultivated—

(1) In homogenous plasma taken from animal 7, now twelve days old.

(2) In that taken from animal 8, now seven days old.

(3) In that taken from animal 9, now three days old.

(4) In fresh autogenous plasma.

In the plasmata from animals 7 and 8 there was no growth at all. In the plasma from animal 9 100 per cent. of the pieces grew and the growth of these was very well marked. In the fresh autogenous plasma 44 per cent. grew. The growth of these was much less marked than that of those grown in the three-day homogenous plasma.

These results are shown in the following table:—

Plasma.	Animal 7.	Animal 8.	Animal 9.	Animal 10.
7	Fresh 42 per cent.	5 days, 100 per cent.	9 days. 60 per cent.	12 days, 0
8	—	Fresh, 13 per cent.	4 days, 67 per cent.	7 days, 0
9	—	—	Fresh, 44 per cent.	3 days, 100 per cent.
10	—	—	—	Fresh, 44 per cent.

It was clear that the increase in the amount of growth which took place when the plasma had been kept frozen for about three days was very marked, thus while autogenous plasma when fresh gave a growth in from 13 per cent. to 40 per cent. of cases, it gave a growth in 100 per cent. of the trials when it had been kept for three days and was homogenous. The fact that some plasmata are good and others bad is also clearly shown by the table. For instance, the plasma of animal 8 is definitely not so good as that of animals

7 and 9. The results obtained in Experiment 9 are specially of interest, for with plasma nine days old growth was obtained. This plasma coagulated well but only gave 60 per cent. of positive results as compared with 100 per cent. obtained with the same plasma when it was only five days old. This would seem to show that the diminution of growth which occurred after the plasma had been kept for a certain period was not entirely due to the lack of power of coagulation, a lack which was considered the possible cause of failure in the case of the testicular tissue. Further experiments were carried out to show whether the increase of growth described above was due to the homogenity of the plasma.

Experiment 11.—Thyroid tissue was cultivated in fresh autogenous and homogenous plasmata. Cultures were also made in plasmata eleven and eight days old respectively. As usual no growth took place in the last two groups. In the fresh autogenous plasma growth occurred in 60 per cent. of the cultures, whilst in the fresh homogenous plasma it was present in 40 per cent. and was rather less marked.

The above results were confirmed by cross experiments carried out in the same way as Experiment 5 was conducted in the case of the testicular tissue.

Experiment 12.—Blood was removed from the lateral ear veins of two rabbits, eight and three days before the experiment. Fresh blood was removed at the time of the experiment and the thyroids were taken out from the two animals at the same time. Cultures were made from each thyroid in all six plasmata. In the eight-day plasmata all four groups showed no growth. The thyroid tissue of animal A showed positive results in 100 per cent. of the trials in the three-day-old autogenous plasma, but no growth at all in the plasma of animal B. With the fresh plasma again there was slight growth in 55 per cent. of the cultures in the autogenous plasma of animal A, and no growth in that of animal B. In the case of the thyroid of animal B there was good growth in 100 per cent. of the trials in the three-day-old plasma of animal A, which in this case was homogenous, and no growth in that of animal B. With the fresh plasma there was fair growth in the plasma of animal A in 64 per cent. of the cultures and none in the plasma of animal B. These results are shown in the table on p. 460.

Thus this experiment confirmed what was found in the case of the testicle, namely, that growth was not dependent upon any quality of the cells or upon the fact that the plasma was homogenous or autogenous, but one plasma was bad so that neither tissue would grow in it, whilst the other was good and gave good results.

	Animal A.		Animal B.	
	Plasma A. Autogenous.	Plasma B. Homogenous.	Plasma A. Autogenous.	Plasma B. Homogenous.
8 days	0	0	0	0
3 days	100 per cent.	0	100 per cent.	0
Fresh	50 „	0	64 „	0

Kidney.

Of this tissue 96 cultures were made. The experiments were all carried out by the method of cross growth, which made all the requisite points clear. One such experiment may be quoted as an example.

Experiment 13.—Plasmata were collected in a manner similar to that used in Experiments 5 and 12. The plasmata were respectively eight days old, three days old, and fresh. In the eight-day-old plasma, as usual, no growth took place. In the case of the three-day-old plasmata the kidney of animal A grew well in the plasma of animal B but not at all in the plasma of animal A. The kidney of animal B also grew well in the plasma of animal B and not at all in the plasma of animal A. With the fresh plasmata growth occurred in the case of both tissues in both plasmata, that in plasma B being rather the better. Growth was in all cases less than that in the three-day-old plasma of animal B.

The results of the kidney cultures supported therefore those obtained with testicle and thyroid.

Summary.

1. The extent of growth of tissues *in vitro* is not dependent upon any quality of the cells themselves.

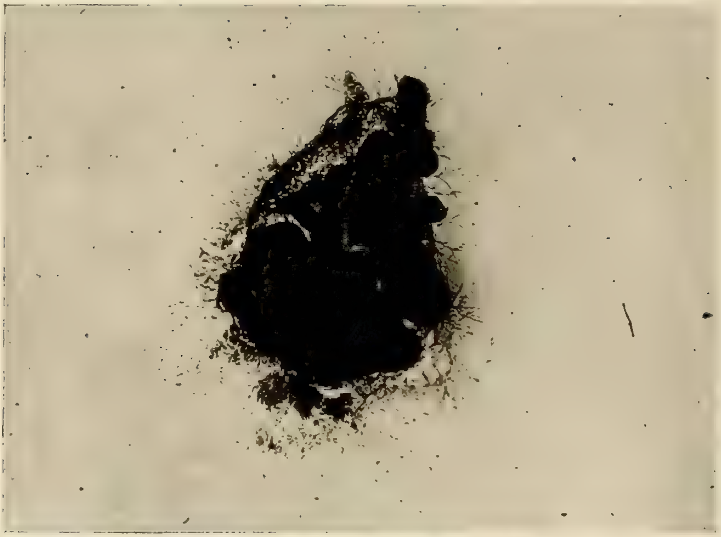
2. The extent of growth varies directly with the character of the plasma.

3. The variation in the plasma does not depend upon whether it is autogenous or homogenous but upon some cause at present unknown.

4. Fresh plasmata appear to contain substances, inhibitory and stimulating, to the growth of cells, the latter being in excess.

5. The inhibitory substance is lessened, or the stimulating substance is increased, by freezing the plasma for one to three days.

6. The stimulating substance is destroyed after the plasma has been frozen for six to eight days.



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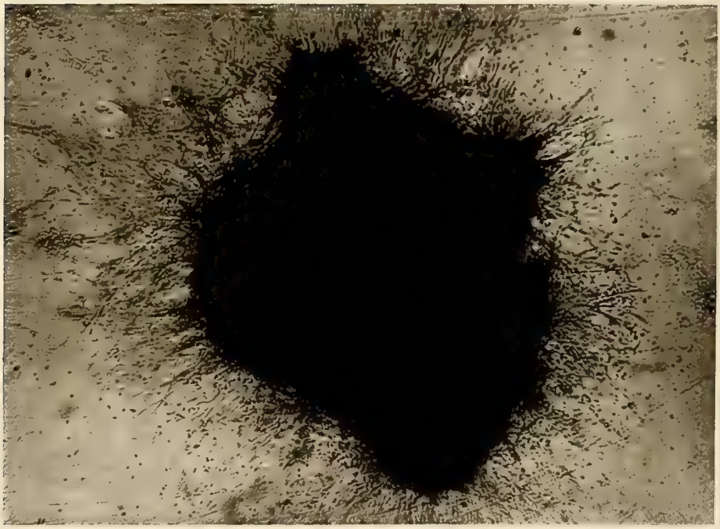

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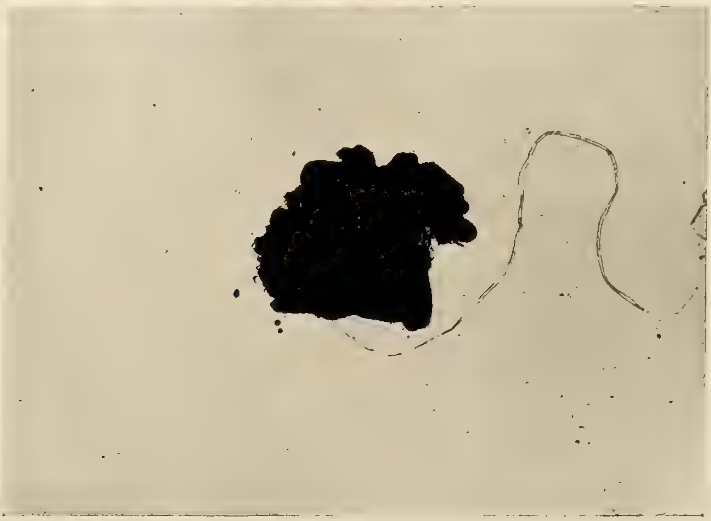

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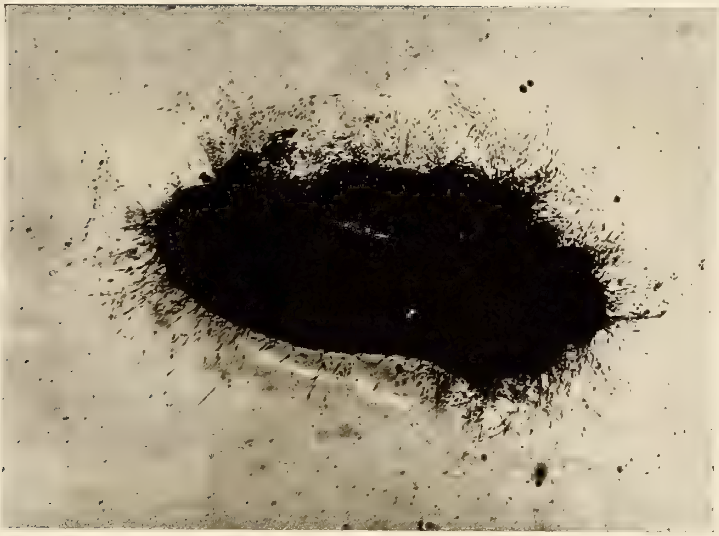
IV.



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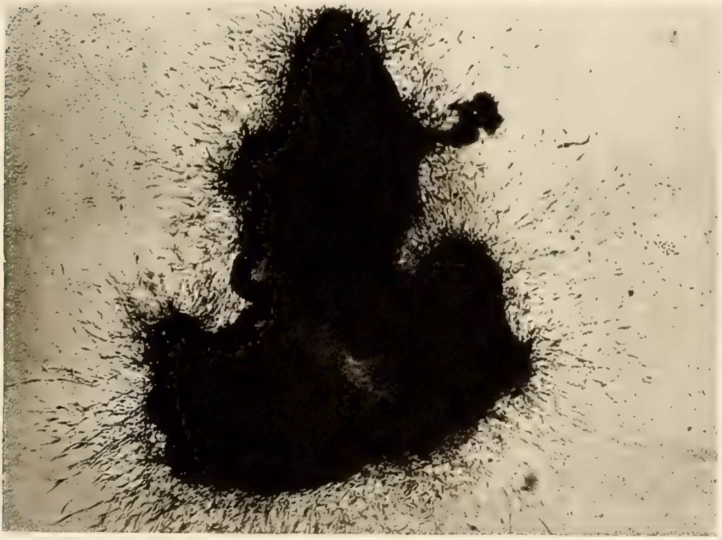

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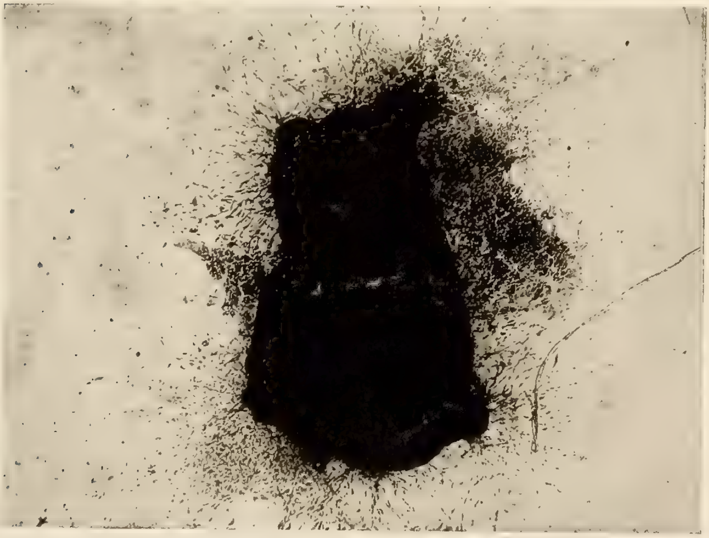


VI.

All x 35.

DESCRIPTION OF PLATES.

1. Three days' growth in fresh homogenous plasma.
2. Three days' growth in homogenous plasma three days old.
3. Three days' growth in homogenous plasma ten days old.
4. Three days' growth in fresh autogenous plasma.
5. Five days' growth in fresh autogenous plasma.
6. Five days' growth in fresh homogenous plasma.

The Decomposition of Formates by Bacillus coli communis.

By Egerton Charles Grey, 1851 Exhibition Scholar.

(Communicated by Dr. A. Harden, F.R.S. Received February 19,—Read March 26, 1914.)

(From the Biochemical Department of the Lister Institute.)

Many observations have been made on the variability of gas production by intestinal bacteria under natural conditions (see Penfold (1911) and Arkwright (1913), where literature is quoted).

Penfold has found that by artificial selection of *Bacillus coli communis* in the presence of sodium chloroacetate, strains may be isolated which produce no gas from glucose and gas in lessened amount from mannitol, although in both cases acid is produced as with the normal organism. The writer has also shown that by artificial selection of *B. coli communis* by the chloroacetate method of Penfold, various stages between the original gas-producing and the selected non-gas-producing strain may be obtained, and the changes have been found to be associated in part with the disappearance of the enzyme which decomposes formic acid (1914). It was found that two kinds of artificially selected strains could be produced from the original strain of *B. coli communis*; one unable to decompose formic acid, and the other still able to bring about this decomposition provided glucose were present. The artificially selected organism, which could not decompose formates even in the presence of glucose, was likewise unable to produce gas from mannitol, whereas the organism which still retained the power of decomposing formates was also able to produce gas from mannitol, although it produced this gas in an amount approximately equal to one-half of that produced under the same conditions by the original *B. coli communis* from which it was derived. It seemed, therefore, likely that by a closer study of the manner in which formic acid is decomposed by the natural and artificially selected varieties of intestinal bacilli it might be possible to gain information concerning the mechanism of the change brought about in the organism by growth on chloroacetate agar which leads to the selection of strains in some cases unable to decompose formic acid and in other cases unable to produce it to the same extent as the normal strains from which they have been derived.

It seemed also of importance to determine what use the decomposition of formic acid might be to the organism. Pakes and Jollyman (1901) and Harden (1901) have shown that *B. coli communis* is capable of decomposing a considerable amount of sodium formate, and that if a small quantity of glucose be added, the amount of hydrogen produced over and above that which could be derived from the glucose added is far greater than the amount produced in the absence of the sugar.

The writer has employed an artificially selected strain of *B. coli communis* obtained by the chloroacetate method; this strain produced in three days no gas from sodium formate peptone water, and only acid but no gas from glucose peptone water, but produced from a mixture of the two sufficient gas to fill the Durham gas tube (length 45 mm.) in 24 hours. The non-production of gas from sodium formate peptone water alone is due, not to the inability of the organism to decompose formic acid, but to the inhibitory action of the alkali due to the natural alkalinity of sodium formate; for if the sodium formate peptone water were acidified with sulphuric acid until the solution imparted a pink colour to litmus, it was found that a small quantity of gas was produced by growth of the artificially selected organism therein for two or three days.

Other sugars and polyhydric alcohols have been employed with similar results, which are discussed under Table II.

By a quantitative study of the decomposition by the bacillus in question of a mixture of glucose and calcium formate, the writer has been able to show that both the amount of glucose and that of formate decomposed is increased (Table III), and there can be little doubt that the formate and sugar are mutually helpful, in that the alkali produced by the decomposition of the former and the acid produced from the latter by neutralising one another maintain that approximately neutral condition of the medium which, as has been proved, is most favourable for the action of the organism.

EXPERIMENTAL.

The Examination of the Behaviour of Non-gas-producing Organisms towards Formates as a means of Deciding whether the Organism has been Derived from an Original Gas-producing Strain.

It has been mentioned above that by artificial selection of *B. coli communis* it is possible to obtain strains which do not produce gas from glucose, and that this phenomenon consists in part, in some cases, in a lessened power to decompose formic acid possessed by the selected organism. In the case of the strains examined by Penfold and Harden (1912) the power of decomposing formic acid was in all cases retained by the selected strains, and certain strains examined in the course of this work were found likewise to have retained this power. In the case of one strain, however, the power to decompose formic acid had been entirely lost. It may, therefore, be considered as probable that the strain incapable of decomposing formic acid represents a more advanced stage in the process of selection, and that, therefore, this type would be more permanent in character. Such indeed has proved to be the case, for while the strain which retains the power to decompose formic acid tends to revert in its properties to the parent organism as regards the production of gas from glucose, the other strain, which cannot decompose formic acid, shows no such tendency, although it has been frequently sub-cultured during the course of seven months.

In view of the fact that the more permanent non-gas-producing type of artificially selected strain is unable to decompose formic acid, it may be suggested that the same phenomenon might be exhibited by naturally occurring non-gas-producing organisms, and that in order to decide whether a strain which, at any particular time, does not produce gas has been recently derived from a gas-producing strain, an examination of its behaviour towards formic acid might be of crucial importance.

It frequently happens that organisms isolated from natural sources differ apparently only as regards the power to produce gas from carbohydrates and allied substances, and the question arises as to whether the one organism may have recently been derived from the other. Arkwright (1913), for example, has obtained varieties of *B. acidi lactici* differing in the aforesaid respect, both strains occurring in the same sample of urine, and he was also able to show that in certain cases the non-gas-producing strain could be trained to decompose sodium formate if grown for some time on a peptone water medium containing this salt. The writer has found that the power to produce gas from mannitol may, in some instances, be made to disappear by simply allowing a broth culture of *B. coli communis* to remain unchanged for three months, or by growth of the gas-producing organism anaërobically in peptone solution containing mannitol in the presence of chalk for about one month. At the end of the period described, if a loopful of the culture be plated out on to agar, many of the colonies which grow at 37° will be found to produce no gas when inoculated into mannitol peptone water tubes. This change may be seen from Table I.

Table I.—The Disappearance from *B. coli communis* of the Power to Produce Gas from Mannitol by Continuous Growth of the Normal Organism in Unchanged Cultures.

History of the culture.	Production of gas.	
	Mannitol.	Glucose.
Normal *B. coli* recently isolated, average 46 normal strains	30 mm. gas	21·0 mm. gas.
The above-mentioned normal strains after being kept in unchanged broth 4 months, average 6 strains	25 ,,	22·0 ,,
Kept in unchanged broth 4 months, average 12 strains	12 ,,	20·0 ,,
,, ,, 4 ,, ,, 12 ,,	5 ,,	18·5 ,,
,, ,, 4 ,, ,, 8 ,,	2 ,,	20·5 ,,
,, ,, 4 ,, ,, 9 ,,	Nil	21·0 ,,

The strains described in Table I, which did not produce gas from mannitol, were examined after growth on broth during several sub-cultures and were found not to produce gas from mannitol when inoculated from the broth tubes into mannitol peptone water. Thus the acquired character is inherited for a considerable time under these conditions. It will be seen from the foregoing table that no change has been brought about in the power to produce gas from glucose, and this is also true for dulcitol. Nevertheless, if by simple growth in peptone water *B. coli communis* yields a strain incapable of producing gas from mannitol, it would seem not unlikely that some similar process might, with time, lead to the disappearance of the power to produce gas from glucose, but such has not so far been observed.

In deciding whether an organism possesses the formic acid decomposing enzyme, which it is suggested here should be used as a criterion of a gas-producing organism, it is not convenient or sufficient to observe whether gas is produced from peptone water containing sodium formate. The test should be made with a mixture of sodium formate and glucose in such proportions that the sodium carbonate which will result from the decomposition of the formate will be approximately sufficient to neutralise the acid which will be produced from the carbohydrate. A convenient mixture is 1·5 per cent. carbohydrate and 0·5 per cent. sodium formate in 1 per cent. peptone water. It will be found under these circumstances that whereas an organism may give only a few bubbles, or even no gas at all, from sodium formate peptone water alone, and none at all from glucose peptone water alone, the mixture may yield gas with great rapidity, so that in 20 hours a Durham tube may be completely filled. This increased gas production is due chiefly to the decomposition of the formate, but partly also to gas which may be produced from the sugar when the solution is maintained neutral, as will be described later.

This increased gas production from formates in the presence of carbohydrates is strikingly illustrated in the case of a selected strain of *B. coli communis* obtained by the chloroacetate method, as will be seen from the following table. The numbers represent millimetres of the tube occupied by gas in the Durham tubes of 45 mm. length.

Table II.—The Effect of Addition of Carbohydrates and Allied Substances on the Decomposition of Sodium Formate by an Artificially Selected Strain of *B. coli communis* producing only a Minute Quantity of Gas from Glucose.

Time.	Sodium formate.	Glucose.	Lactose.	Mannitol.	Dulcitol.	Sorbitol.	Glycerine.
(Concentration of the Sugar or Alcohol 2 per cent.)							
hours.							
12	Nil	Nil	Nil	Trace	Nil	Trace	Nil
24	"	"	"	11	"	12	"
36	"	Minute bubble	Minute bubble	23	"	25	"
60	"	No increase	0·5	No increase	"	No increase	Trace
84	"	"	2·0	"	3	"	1
108	"	"	3·0	"	30	"	1
132	"	"	3·0	"	37	"	1
Evolution of Gas from the above Carbohydrates and Alcohols after Admixture with Sodium Formate. (Carbohydrate or Alcohol 1·5 per cent., Sodium Formate 0·5 per cent.)							
12		12	4·0	Trace	Nil	2	Nil
24		Full	Full	10	"	10	"
36				34	"	Full	"
60				Full	"		5
84					5		7

The following facts should be noted in connection with the experiment described above :—

(1) The non-production of gas from formate peptone water alone was due, in part, to the natural alkalinity of the medium. To demonstrate this varying quantities of N/10 H_2SO_4 were added to a series of sodium formate peptone water tubes, which were then inoculated with a loopful of a broth culture of *B. coli communis.* It was found that in those tubes in which the reaction to litmus was nearest to neutral, there was a slight production of gas, whereas those which were distinctly alkaline or acid showed no gas at all.

(2) The manner in which the inoculation is made is also of importance. Several tubes of sodium formate peptone water were inoculated each with a loopful of a broth culture of *B. coli,* and another set of tubes were inoculated each with a loopful of an agar growth of the same organism. The former set of tubes produced no gas, the latter produced one-tenth of a Durham tube. This difference in the production of gas cannot be due simply to the size of the inoculation, for even when kept for 10 days the formate tubes inoculated from the original broth culture showed no production of gas. Probably, therefore, the bacillus when grown on agar contains more of the formic acid decomposing ferment than when grown in broth.

(3) The decomposition of sodium formate is not assisted in the same degree by mannitol as it is by glucose and the other sugars or by sorbitol, and it may be possible that this phenomenon is related to the fact already mentioned, that the power to produce gas from mannitol disappears from old broth cultures of *B. coli communis,* when these have remained unchanged for some months, and still more readily when the fluid contains mannitol and the products therefrom.

It should be noted also that, since less acid is produced from a hexahydric alcohol than from the same weight of a hexose when fermented by *B. coli communis,* the fact that the alcohol does not assist so well in the acceleration of the decomposition of the formate by the organism is in harmony with the view that it is the neutralisation of the medium by the acid produced by the carbohydrate or allied substance which is of assistance for the further decomposition of the formate.

The fact that in any particular experiment no gas may be produced from glucose peptone water is not a complete proof that an organism cannot produce gas at all from glucose, for the acid produced under circumstances in which no precaution is taken to neutralise the medium inhibits the decomposition of formic acid.

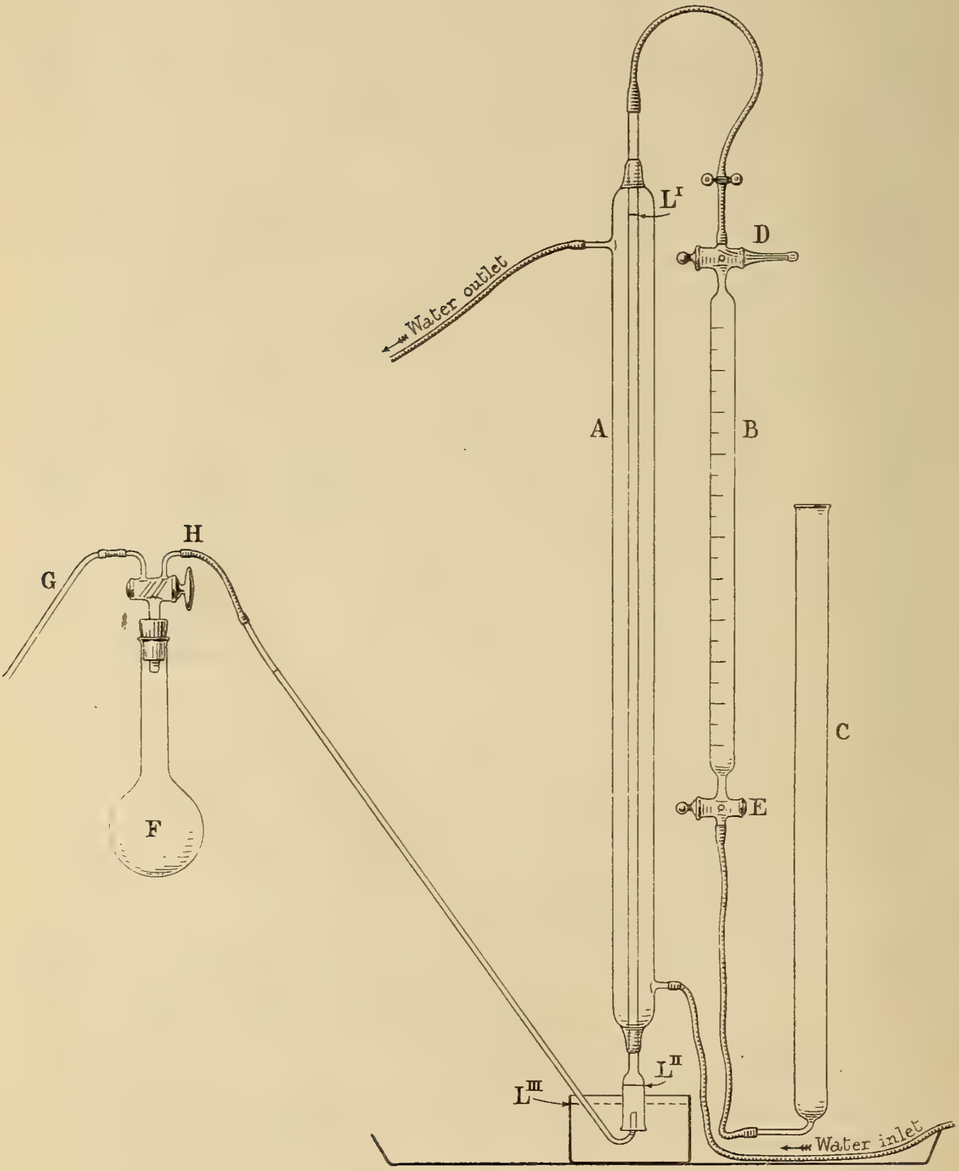
Quantitative Study of the Rate and Extent of Decomposition of Sodium Formate and Glucose by an Artificially Selected Non-gas-producing Strain of B. coli communis *when grown on them either separately or together.*

In order to determine the causes of the greatly increased gas production observed when *B. coli communis* was grown on a mixture of sodium formate and glucose, the change was followed quantitatively. For this purpose it was necessary to determine the weight of formic acid and glucose consumed in the reaction and the total carbon dioxide and acid produced, and also to measure the gas production from time to time. The method which was employed would be suitable for the examination of the decomposition of many other substances by bacteria, and it is therefore described in detail.

A quantity of 50 or 100 c.c. of 2 per cent. glucose in 1 per cent. peptone water is sterilised and inoculated with the organism. The cotton-wool plug, which should fit loosely, is pushed half-way down the neck of the flask, and the flask is connected with a Schiff's gas burette by means of a rubber stopper provided with a two-way tap. The burette, which is filled with mercury, is in connection with a reservoir for adjusting the pressure, as in the apparatus described by Harden, Thompson, and Young (1910). Before beginning the experiment, air may be removed from the flask by putting it in connection with the burette. On lowering the reservoir air passes into the burette. Nitrogen is then admitted to the flask by reversing the tap, and this process is repeated four or five times, when the oxygen will have been practically all removed. The flask is well immersed in a water-bath maintained at 37°. When it is desired to stop the reaction, the flask is removed from the water, and the contents are, after turning the two-way tap so as to put the flask in connection with the apparatus described below, carefully brought to the boil, the gas driven out displacing the mercury from the inner tube A (see figure).

Details of the Use of the Gas Collecting Apparatus.—The object of the apparatus is to collect all the gases which remain in the fermentation flask both free above the surface of the medium and dissolved in the fluid.

A is an ordinary Liebig's condenser set vertically and connected by a three-way tap D with a gas burette B accurately graduated. By putting D in connection with the pump or by raising the tube C, which must be filled with mercury, the mercury rises to fill B; the tap E is then closed. The tap D may now be reversed and mercury drawn up into the inner tube A from the reservoir L‴ to the level L′. A circulation of water in the Liebig's condenser is not necessary for the condensation of the steam, but helps in keeping the temperature of the collected gas constant. To collect the gases the flask F is heated carefully and the contents brought to the boil; the gas displaces the mercury from the inner tube A, and should the gas evolved be more than sufficient to fill A the tap D may be turned so as to connect



A and B, and the tap E turned so as to connect B and C, while C is lowered; the mercury in B falls with a corresponding rise of mercury in A.

The volume of the inner tube from a definite etched mark L'' to the tap

D, including the volume of the pressure tubing connecting A and B, having been previously determined, the total volume of evolved gases may be measured by raising the reservoir C, E being open, and D turned to connect A and B; the mercury then rises in B and falls in A, in which it is allowed to fall to the level L″. To correct for pressure an allowance may be made for the height of the mercury from the surface of the reservoir L‴ to L″, but it is also quite convenient to lower the whole Liebig's condenser until L″ coincides with L‴. The volume of gases in the graduated tube B is then observed, and this volume added to that of the inner tube A. A sample of the gases may now be conveniently removed by lowering C. When B contains sufficient of the gases for analysis, the whole apparatus B–C may, if desired, be removed from its connection with A.

The apparatus has been described in detail because it is of use for the determination of all gases remaining in the fermentation flask. In the experiments recorded in the present communication, however, it was only of value to determine residual carbon dioxide.

Details of the Determinations.—The carbon dioxide boiled off from the solution, as described above, is measured in the usual way. The flask is now detached from the apparatus and the contents filtered from the deposit of chalk, and the filtrate and washings precipitated in hot solution with ammonium oxalate. The precipitate of calcium oxalate is used to estimate the calcium corresponding to the total acids produced during the fermentation, an allowance being made for the calcium in the peptone. The filtrate from the calcium oxalate is acidified with oxalic acid and distilled in steam, the distillate neutralised with deci-normal potash and evaporated to dryness; the residue is dissolved in about 50 c.c. of water, and the formic acid determined by the reduction of mercuric chloride. The residue from the steam distillation is made up to a definite volume, and an aliquot portion used for the determination of the residual sugar by Bertrand's method after the removal of peptone by Patein's mercuric nitrate reagent.

The results of the experiment are summarised in Table III.

It will be seen from Table III that about ten times as much gas was produced by the selected strain of *B. coli communis* from calcium formate in the presence of glucose as was produced by it from calcium formate alone. The amount of sugar decomposed in the presence of calcium formate is considerably greater than in its absence, even when the medium is kept as far as possible neutral by chalk.

Table III.—Comparison of the Action of an Artificially Selected Strain of *B. coli communis* (Escherich) on Glucose alone; Glucose + Calcium Formate; Calcium Formate alone.

	Conditions of the experiment.			
	Glucose alone. Medium not neutralised during fermentation.	Glucose alone. Medium kept neutral by chalk.	Glucose + calcium formate + chalk.	Calcium formate alone.
Duration	99 hours	99 hours	120 hours	120 hours
Glucose before ...	3·385	1·6926	1·6926	None
„ after	3·2276	1·0628	None	„
„ consumed	0·1574	0·6098	1·6926	—
Formic acid before	None	None	0·5244	0·5244
„ „ after	0·0874	0·0249	0·0276	0·4988
„ „ consumed	—	—	0·4968	0·0256
CO_2 total gas	42 c.c.	96 c.c.	291 c.c.	12 c.c.
CO_2 from acids on chalk	41 „	90 „	161 „	—
CO_2 from formate	—	—	119 „	12 c.c.
CO_2 from sugar ...	—	6 c.c.	11 „	—

The medium contained in all cases 1 grm. peptone (Witte) in 100 c.c.

The actual carbon dioxide produced by the organism from calcium formate is in reality twice that actually evolved, for in the decomposition

$$(HCOO)_2Ca + H_2O = CaCO_3 + CO_2 + 2H_2$$

it is clear that one-half of the CO_2 is retained in combination with the calcium.

These results bring out, therefore, very clearly one object which is attained by the decomposition of formates by these bacteria, viz.: that the organisms are thereby supplied with the best possible neutralising agent. For the formate by being decomposed into carbon dioxide and hydrogen virtually liberates alkali within the bacterial cytoplasm, and thus not only neutralises the medium, but also the bacteria themselves. Moreover the calcium formate being itself neutral possesses none of the disadvantages which would arise from the presence of even a slight excess of alkali. It would be difficult to devise a more efficient means for maintaining neutrality in this case. I would suggest the utilisation of sodium or calcium formate as a neutralising agent in working with those organisms capable of decomposing it, especially for solid media, with which the addition of dissolved alkali from time to time would be impracticable.

Summary and Conclusion.

(1) The power of *B. coli communis* to decompose formic acid varies considerably when the organism has been kept for some time on artificial media.

(2) The decomposition of formates is inhibited by a very small excess of either acid or alkali and, therefore, a greatly increased decomposition of formates results if glucose is added, since the acid produced from the sugar neutralises the alkali from the formate.

(3) A method and apparatus are described by which the decomposition of various substances by micro-organisms may be followed quantitatively requiring only 50–100 c.c. of the solution.

(4) It has been suggested that in place of a solution of sodium formate a mixture of sodium formate 0·5 per cent. and glucose 1·5 per cent. should be used as a test of a gas-producing strain, since by this means the production of gas from formate is greatly increased, and it is also suggested that the test could be used as a criterion as to whether an organism, which has been recently isolated from some natural source and produces no gas from glucose peptone water, may be regarded as having been recently derived from a gas-producing strain.

(5) It has been shown that formates may be conveniently used as neutralising agents, and that thereby the activity of gas-forming organisms may be considerably increased.

In conclusion I would express my thanks to Prof. Harden, F.R.S., for help and criticism.

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The Enzymes which are Concerned in the Decomposition of Glucose and Mannitol by Bacillus coli communis.

By Egerton Charles Grey, 1851 Exhibition Scholar.

(Communicated by Dr. A. Harden, F.R.S. Received February 19,—Read March 26, 1914.)

(From the Biochemical Department of the Lister Institute.)

By the cultivation of bacteria in the presence of certain substances, for the most part toxic in character, it is possible to obtain strains in which the fermentative powers differ considerably from those of the parent organisms. As an example may be taken a variety of *B. coli communis* (Escherich) which was produced by the growth of that organism on agar containing sodium chloroacetate (see Penfold, 1911). This strain differed from the parent strain in that it now decomposed glucose with the production of acid but not of gas.

This result pointed to two possibilities; firstly the decomposition of glucose by the selected strain might be brought about by a set of ferments, which acted very differently from those of the normal strain responsible for the decomposition of the same substance, or secondly the primary cleavage products of glucose might be the same both from the original and the selected strain, and the difference between the action of the two might depend upon some secondary process, as for example the decomposition of formic acid, through which, as Pakes and Jollyman (1901) and Harden (1901) have shown, the carbon dioxide and hydrogen most probably arise.

It is obviously of great biological importance to know whether the changes brought about by growth on sodium chloroacetate result in any profound modification in the carbohydrate metabolism of the organism. It was, at the outset, considered most probable that those enzymes which were responsible for the cleavage of the glucose molecule into its primary products would be less likely to be lost than those which brought about the secondary changes.

It was hoped, therefore, that by a comparison of the products formed from glucose and mannitol by the normal organism with those produced from the same substances by the artificially modified strains it would be possible to determine how many different enzymes were concerned in the process.

If a number of products are formed by one enzyme the ratio which they bear to one another should not be altered by the process of selection, or conversely if on selection the ratio between any two substances is found to alter it may be taken as evidence that these two substances are not produced by one enzyme, unless these two substances can replace one another to a certain extent owing to secondary reactions.

Isolation of the Organism.

B. coli communis (Escherich) was chosen for this work since the first observations made by Penfold (1911) on the disappearance of the gas-producing power by growth in the presence of sodium chloroacetate were made with this organism. It was found, however, that very many strains of *B. coli communis* could be isolated, showing not only differences in degree (which need not be considered here), but also of kind.

The organisms were isolated from human fæces in the ordinary way. A broth culture was made and from this bile salt cane-sugar neutral red agar plates were inoculated; after incubation for one or two days at 37°, a number of white colonies (cane-sugar non-fermenters) were removed to tubes containing lactose peptone water coloured with litmus, and provided with Durham gas tubes. Those tubes which on incubation produced acid and gas (lactose fermenters) were used to inoculate a series of tubes containing dulcitol peptone water. By these three operations organisms were obtained which according to MacConkey (1905) belonged to the *B. coli communis* (Escherich) group. The general characteristics of the four varieties which were found will be seen by reference to Table I.

Table I.—Characters of Strains of *B. coli communis* occurring together in Normal Fæces.

Motility.	Indole production.	Milk clotting.	Fermentation of sugars, etc.			
			Glucose.	Lactose.	Mannitol.	Cane-sugar.
1. Rapid	Strong	24 hrs.	A, G	A, G	A, G	Nil
2. Slight	Medium	24 ,,	A, G	A, G	A, G	Nil
3. Slight	Strong	5 days	A, G	A, G	A, G	Nil
4. Rapid	Nil	24 hrs.	A, G	A, G	A, G	Nil

A = acid. G = gas.

All the above strains were Gram negative, did not liquefy gelatin and did not give the Voges and Proskauer reaction. Very many examinations were made of the motility in from 3 to 10 hours' cultures.

The most striking difference is that between the rapidly motile No. 1 and the practically non-motile No. 2. These were chosen, therefore, for further study, since it seemed possible that the motile organism might differ considerably in its metabolism from that which was slightly motile.

The consideration of this relationship is, however, not one of the objects of the present communication.

It is important to note that the difference in motility between strain No. 1 and No. 2 is not merely one of degree but rather one of kind. It is, as a matter of fact, rather difficult to decide whether No. 2 is really motile at all, and only after concentrating the attention on one bacillus and observing its position from time to time in relation to an adjacent organism is it possible to decide that it really does exhibit a motion of translation. The strain was examined very many times in cultures from 3 to 12 hours' growth and at later periods, but no increase in the motility of this strain was ever observed. With the strain No. 1 the appearance is entirely different; in cultures of any age from 3 to 24 hours, rapid motility is readily observed. In cultures less than 9 or 10 hours' old the bacilli may be seen travelling with such rapidity that it is almost impossible to follow the course of any one particular bacillus. In young cultures (3 to 7 hours) the bacilli may be readily seen in long threads, in which the bacilli have not had time to separate. No such threads were obtained with strain No. 2.

The highly motile typical *B. coli communis* (Escherich) will be referred to as No. CI, and the feebly motile strain as No. CF.

Artificial Selection of Non-gas-producing Strains by Growth of B. coli communis *(Escherich) on Agar containing Sodium Chloroacetate.*

The technique of the chloroacetate method of selection has been described by Penfold (1911) and has been closely followed here. It has been found, however, that there is very considerable variation in the power of resistance to sodium chloroacetate, and also in the appearance of the chloroacetate agar plates inoculated with various strains of *B. coli* (Escherich). The nature of the changes brought about by growth in the presence of sodium chloroacetate will be discussed in a separate communication, and it must suffice to say here that the changes do not merely consist in the simple disappearance of the power to produce gas from glucose, but are, rather, of such a nature as to affect, to a greater or less extent, most of the enzymatic functions of the cell.

Some of the selected organisms are grown anaërobically only with great difficulty, and hence their chemical products cannot be readily investigated. Other strains show the property of spontaneously agglutinating and cannot, therefore, be very well shown to be derived from the original organism. In this work, only those selected strains which, by means of the agglutination test, could be demonstrated as related to the original organisms, have been employed for the examination of the decomposition products from glucose and mannitol. Two varieties of selected organisms have been used—the one (CI selected) produced from the typical *B. coli* (Escherich) No. CI, like the organism of Harden and Penfold (1912), produced acid and no gas from glucose, while it still produced gas from mannitol, and also retained the power of decomposing formates in the presence of glucose (see Grey, 1914). The other (CF selected), obtained from the organism CF, now gave acid but no gas, either from glucose or from mannitol, and was also unable to decompose formates even in the presence of glucose. This second selected organism might perhaps be regarded as representing a further stage of selection than the first, but I have not found it possible so far to obtain from CI a strain comparable to CF (selected).

The Relationship between the Normal and the Artificially Selected Strains as established by the Agglutination Reaction.

The artificially selected strains (when made into an emulsion by the addition of normal saline to an agar growth) were found in many cases to agglutinate spontaneously. The freshly selected strain was therefore first plated out on plain agar and agar slopes made from several individual colonies. By this means it was found possible to obtain strains which did and strains which did not agglutinate spontaneously. The latter were then treated with rabbit serum containing the specific agglutinins for the normal strains CI and CF, and it was found that the serum obtained by inoculating a rabbit with normal CI agglutinated normal CI and the artificially selected

Table II.—Demonstration of the Relation between the Normal and Artificial Selected Strains (obtained by the Chloroacetate Method) by means of the Agglutination Test.

Bacterial emulsion made with—	Dilution of the serum.									
	1/100.	1/200.	1/400.	1/800.	1/1600.	1/3200.	1/6400.	1/12800.	1/25600.	1/51200.
Serum obtained with CF normal.										
CF (normal)	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +	–
CF (selected)......	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +	–
CI (normal)	–	–	–	–	–	–	–	–	–	–
CI (selected)	–	–	–	–	–	–	–	–	–	–
Serum obtained from CI normal.										
CI (normal)	+ + +	+ + +	+ + +	+ + +	+	–				
CI (selected)	+ + +	+ + +	+ + +	+ +	+	–				
CF (normal)	–	–	–	–	–					
CF (selected)......	–	–	–	–	–					

strain derived from it, but did not agglutinate the strain CF. And, likewise, the serum obtained by inoculating a rabbit with the normal strain CF agglutinated the normal strain CF, and the selected strain derived from it (CF selected) up to a dilution of 1 : 25600, but did not produce the slightest agglutination with the normal or selected strain No. CI.* This may be seen from Table II.

Analytical Technique.

The methods of analysis described by Harden (1901) have been for the most part closely followed; certain slight modifications, however, have been introduced, which may be described here.

Volatile Acids.—In the steam distillate which is used for the determination of formic and acetic acid, the formic acid has been determined by the formation of mercurous chloride, and the acetic acid obtained by subtracting the amount of formic acid so found from the total acids determined previously by titration of the whole distillate with alkali, using phenolphthalein as indicator. Two errors are introduced here due to the presence of small amounts of carbonic acid and lactic acid in the distillate. The carbonic acid has, therefore, been estimated by barium hydroxide and the lactic acid by Ryffel's method. This estimation of lactic acid in the distillate becomes of importance when the amount of acetic acid is small.

The distillation to obtain the volatile acids was carried out in two stages. The first fraction was obtained without admitting steam, measured about 400 c.c., and contained the alcohol and part of the volatile acid. This fraction was titrated with standard baryta solution. A slight excess of baryta was then added, and the solution distilled with a fractionating column in order to remove the alcohol. The residual fluid now contained a granular precipitate of barium carbonate, which was removed by rapid filtration and titrated at the boiling point with N/10 H_2SO_4. The barium hydroxide corresponding to the barium carbonate was deducted from that required to neutralise the first distillate. In this way an accurate correction may be made for the carbon dioxide dissolved in the distillate.

After removal of the first 400 c.c. steam was admitted, and the distillation continued until 100 c.c. of the distillate required only 0·1–0·2 c.c. of normal alkali for neutralisation. The total steam distillate usually measured about 2500 c.c. The distillate was neutralised with potash, united with the first fraction, and the whole evaporated to dryness. The residue was dissolved in 100 c.c. of water, and an aliquot portion used for the determination of formic acid, another portion being used for lactic acid by Ryffel's method (1909).

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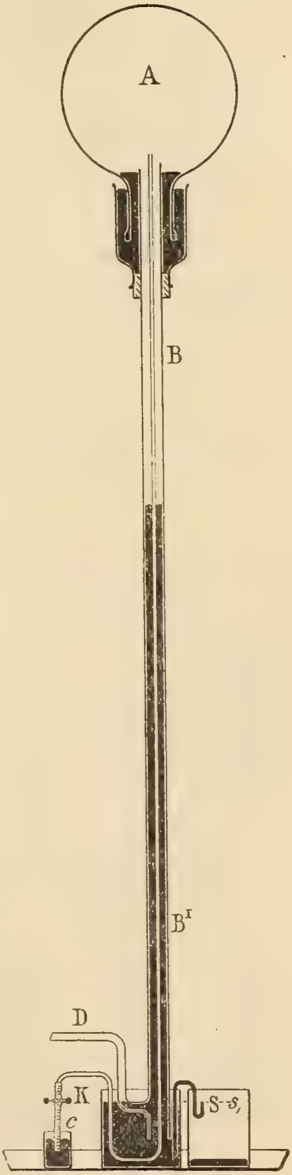
* The agglutinating sera were kindly prepared for me by Dr. J. A. Arkwright of this Institute, to whom my best thanks are due.

The extent of the correction for carbonic acid and lactic acid in the distillate of volatile acids may be seen from the figures quoted in the table on p. 478.



Collection of the Gas.

The carbon dioxide and hydrogen evolved were in some experiments collected in the apparatus of Harden, Thompson, and Young (1910); in other cases a simplified form of this apparatus was employed, which is here figured. The object of this modified form of apparatus is to dispense with all taps and to reduce the number of glass joints. The present form of apparatus has but one glass junction, and has also the advantage that when evacuated it can be sealed by allowing mercury to rise in the inner capillary tube through which the air has been pumped out of the flask. The arrangement for maintaining the pressure in the fermentation flask constant (by adjusting the level of the surface of the mercury in the reservoir automatically) is also of a simpler type.

The flask A is evacuated by means of the capillary tube, which passes up through the tube B in which mercury rises as the air is removed. The tube from the fermentation flask in the incubator is attached to D by a rubber junction. The gas in the fermentation flask is evolved under atmospheric pressure, this equalisation of the pressure in the flask with that of the atmosphere being effected by means of the S-shaped syphon (*s*), which is filled with mercury and automatically adjusts the level of the surface of the mercury in the mercury reservoir.

By plunging the rubber tube *c* beneath mercury and opening the clip K mercury may be allowed to rise in the capillary tube, and thus the flask A becomes completely sealed from the atmosphere.

The neck of the flask A may be plunged beneath wax. This substance although quite effective, is somewhat troublesome to use, owing to shrinkage on cooling. A rubber stopper plunged beneath mercury is, on the whole, a simpler means of sealing off the flask.

Total volatile acid, c.c. normal potash.	Carbonic acid, c.c. normal baryta.	Lactic acid. c.c. normal (Ryffel's method).
48·5	1·44	1·68
38·2	1·0	1·1
34·3	0·3	0·8
51·5	1·0	1·0
41·1	1·3	0·6

Results of Analysis of the Decomposition Products of Glucose and Mannitol formed by the Action of the Normal and Selected Strains of B. coli communis.

Table III.

Typical *B. coli communis* (rapidly motile) No. CI.						
Product.	Normal.				Selected.	
	per cent.	per cent.	Mean.			
		On Glucose.				
CO_2	14·90	14·74	14·82	18·66	2·25	17·23
H_2	0·55	0·52	0·54		0·08	
Formic	3·24	3·36	3·30		14·90	
Acetic	14·10	12·91	13·00		5·69	
Lactic	39·42	36·91	38·07		59·60	
Succinic	3·60	4·60	4·20		5·50	
Alcohol	12·83	11·02	11·93		4·90	
			85·86		92·92	
Ratio $CO_2 : H_2$...	1·23	1·29	1·26		1·28	
		On Mannitol.				
CO_2	26·66	28·00	27·33	35·77	13·33	31·45
H_2	1·04	1·06	1·05		0·64	
Formic	7·21	7·56	7·39		17·48	
Acetic	7·33	6·75	7·04		7·20	
Lactic	22·82	26·27	24·55		19·95	
Succinic	8·80	5·00	6·90		8·61	
Alcohol	27·06	26·85	26·95		27·46	
			101·21		94·67	
Ratio $CO_2 : H_2$...	1·17	1·20	1·19		0·95	

Table III—*continued.*

Variety *B. coli communis* (very slight motility) No. CF.				
	Glucose.		Mannitol.	
	Normal.	Selected.	Normal.	Selected.
	per cent.	per cent.	per cent.	per cent.
CO_2	16·92	None	38·50	None
H_2	0·42 } 27·07	None } 11·80	1·45 } 41·52	None } 32·50
Formic	9·73	11·80	1·57	32·50
Acetic	18·49	10·13	12·88	11·20
Lactic	36·83	62·00	7·48	15·84
Succinic	0·74	0·80	5·60	6·20
Alcohol	18·06	5·30	26·56	22·89
	101·19	90·03	94·04	88·63
Ratio $CO_2 : H_2$...	1·83		1·21	

These results may also be expressed as carbon atoms per molecule of glucose and mannitol respectively.

Table IV.

Product.	CI (rapidly motile).		CF (slightly motile).	
	Normal.	Selected.	Normal.	Selected.
	per cent.	per cent.	per cent.	per cent.
	Glucose (carbon atoms per molecule).			
CO_2	0·60	0·10	0·70	—
Formic	0·14	0·55	0·40	0·46
Acetic	0·81	0·34	1·11	0·61
Lactic	2·37	3·60	2·20	3·72
Succinic	0·22	0·27	0·04	0·04
Alcohol	0·90	0·39	1·41	0·40
	5·04	5·25	5·86	5·23
	Mannitol (carbon atoms per molecule).			
CO_2	1·09 } 1·37	0·54 } 1·23	1·58 } 1·64	—
Formic	0·28	0·69	0·06	1·27
Acetic	0·44	0·45	0·78	0·67
Lactic	1·48	1·20	0·45	0·95
Succinic	0·36	0·45	0·34	0·38
Alcohol	2·11	2·15	2·07	1·86
	5·76	5·48	5·28	5·13

Discussion of Results.

The most significant fact in connection with these results is that whereas in their action on glucose, the artificially selected strains of *B. coli communis* have been considerably modified, in their action on mannitol the only important change is the non-decomposition in the one case, and only partial decomposition in the other case, of formic acid into carbon dioxide and hydrogen.

The results with mannitol present greater uniformity than those with glucose, and may be conveniently considered first. It will be seen that the ratio (alcohol + acetic acid)/2 : formic acid* is practically constant and almost equal to unity. Thus

$$\frac{2\cdot55}{2\times1\cdot37}=0\cdot93;\quad \frac{2\cdot6}{2\times1\cdot23}=1\cdot05;\quad \frac{2\cdot85}{2\times1\cdot64}=0\cdot87;\quad \frac{2\cdot53}{2\times1\cdot27}=1\cdot00.$$

This relationship also holds good for the earlier analyses of Harden (1901), and points to two conclusions—

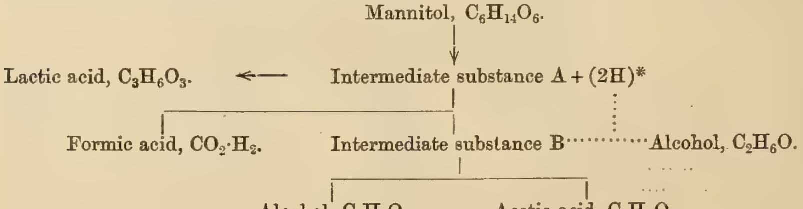
(1) Alcohol and acetic acid are probably derived from an intermediate substance common to them both, and they may therefore, to a certain extent, replace one another. (2) This intermediate substance from which alcohol and acetic acid are produced is itself formed in constant ratio to formic acid.

Lactic acid might be regarded as being formed directly from mannitol by the action of a special enzyme, but this could only occur if (*a*) hydrogen were evolved in excess of carbon dioxide, or (*b*) alcohol and formic acid were produced by the same enzyme which produced lactic acid, as, for example, in accordance with the equation

$$C_6H_{14}O_6 = C_3H_6O_3 + C_2H_5OH + HCOOH.$$

But if such a change as is represented by this equation were effected in one step by a single enzyme then, since the proportion of lactic acid actually produced is only one-third of that demanded by this equation (see Table IV), it would follow that there must be another origin for alcohol and formic acid.

The following hypothetical schemes are put forward to represent the decomposition of mannitol and glucose :—


Mannitol, $C_6H_{14}O_6$.
Lactic acid, $C_3H_6O_3$.
Intermediate substance A + (2H)*
Formic acid, $CO_2 \cdot H_2$.
Intermediate substance B
Alcohol, C_2H_6O.
Alcohol, C_2H_6O.
Acetic acid, $C_2H_4O_2$.

* Hydrogen is here written as atomic hydrogen to indicate that it is intramolecular.

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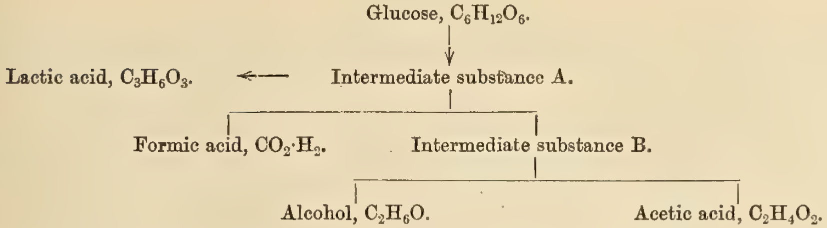
* Formic acid includes free carbon dioxide and hydrogen.


Glucose, $C_6H_{12}O_6$.
Lactic acid, $C_3H_6O_3$.
Intermediate substance A.
Formic acid, $CO_2{\cdot}H_2$.
Intermediate substance B.
Alcohol, C_2H_6O.
Acetic acid, $C_2H_4O_2$.

The intermediate substance A is unknown, but is postulated to account for the formation of lactic acid in such a way that the enzyme which produces lactic acid from glucose may also produce lactic acid from mannitol. The substance is probably related to pyruvic aldehyde.

The intermediate substance B from which it is suggested that alcohol and acetic acid are derived is probably acetaldehyde. This view is supported by the evidence that acetaldehyde may be detected among the products of decomposition of glucose by *B. coli communis* (Grey, 1913).

Two molecules of acetaldehyde might undergo the Cannizzaro reaction (Parnas, 1910) with the production of alcohol and acetic acid, thus

$$2CH_3{\cdot}CHO + H_2O = CH_3{\cdot}COOH + CH_3{\cdot}CH_2{\cdot}OH.$$

If this were the main change in the case of glucose, it would account for the production of alcohol and acetic acid in approximately equimolecular proportions.

Again, acetaldehyde might be reduced to alcohol as postulated by Kostytscheff (1912) for alcoholic fermentation by yeast or directly oxidised.

In the case of mannitol this reduction might be of great importance. It is represented by the dotted lines in the scheme. And since, in this case the whole, or nearly the whole, of the hydrogen formed, along with the intermediate substance A, would be available for this purpose, the result would be the production of alcohol in large excess over that of acetic acid, which is actually observed.

While, however, the decomposition of mannitol and glucose may thus be represented as occurring along the same general lines, it is clear that some essential difference must exist between the mechanisms of the two reactions, or they would not be so differently affected by the process of selection on chloroacetate agar.

The simplest supposition is that this difference affects the production of formic acid and intermediate substance B, for artificially selected organisms produce these substances from glucose in greatly diminished amount, whereas from mannitol their production is not seriously altered.

While the exact nature of the difference in the two mechanisms must still be a matter of conjecture, it may with some probability be supposed that it is connected with the presence in the products from mannitol of hydrogen available for reduction. It must be remembered that the two hydrogen atoms possessed by mannitol in excess of those present in glucose are only capable of reducing half the possible amount of B which could be produced from one molecule of mannitol. Hence, even if half the mannitol were converted into lactic acid, these extra hydrogen atoms could be completely taken up by B. As a matter of fact not more than one-quarter of the mannitol appears as lactic acid, so that a considerable part of B is reduced to alcohol and the remainder probably undergoes the same change as in glucose, forming equimolecular proportions of alcohol and acetic acid.

It must be noted that from the above considerations one would expect that the production of acetic acid from mannitol by the selected organism would be somewhat less than by the normal. In my figures, however, this is not demonstrated to be the case, but it must be remembered that the amount of acetic acid produced by the selected organism does not exceed that produced from glucose.

In the absence of more experimental results, however, it would be premature to discuss other possible origins of acetic acid.

In the scheme for the decomposition of mannitol the production of the excess of alcohol, as compared to the case of glucose, is represented as occurring through the agency of this extra hydrogen.

In the case of glucose, on the other hand, alcohol can only be produced if there be simultaneously the formation of some oxidation product, or in other words the hydrogen would have to be supplied by a reductase.

It should be remembered that the aldehydomutase of Cannizzaro which brings about the conversion in this case of two molecules of acetaldehyde into acetic acid and ethyl alcohol is in reality also a reductase, the acceptor for the oxygen being the same as the substance reduced. The essential difference between the two changes would then reside in the necessity for the co-operation of a reductase in the decomposition of glucose which would not be required to the same extent for that of mannitol.

In all other respects after the preliminary decomposition of the original molecule the two actions would then require exactly the same enzymes.

Considered dynamically, the reaction by which the intermediate substance A changes into formic acid and substance B occurs more rapidly with mannitol than with glucose, so that in the final products less lactic acid is formed in the case of mannitol than in the case of glucose.

This acceleration of the reaction in the case of mannitol by which intermediate substance A yields ultimately formic acid and alcohol as chief products, may be due to the reduction of substance B to alcohol whereby it is removed from the sphere of the decomposition of A.

If, then, the reductase were to be diminished as the result of selection on chloroacetate agar, the removal of B from the sphere of decomposition of A would be slower. The decomposition of A into more of B and formic acid would therefore be specifically hindered, and as a result the production of lactic acid relatively increased.

This is what is actually observed. On the other hand the decomposition of mannitol would be unaffected, as is also found to be the case.

The view that the artificially selected strain produced by growth on chloroacetate agar is deficient in some reducing mechanism is further supported by the fact that many of these strains show diminished power of growing anaërobically. Moreover it might be expected that this method of selection would lead to the survival of a strain deficient in reductase, for a strain with a highly developed reducing mechanism would probably convert monochloracetic acid to acetic acid with the liberation of hydrochloric acid, which would certainly not be of advantage to the organism. Such a process might therefore lead to the survival of the strain in which the reducing mechanism was poorly developed.

Summary and Conclusions.

Two artificially selected strains of *B. coli communis* obtained by growth of normal *B. coli communis* (Escherich) on agar containing sodium chloroacetate have been examined quantitatively as regards their action on glucose and mannitol. In both cases the selected strains produced from glucose, lactic acid in relatively greater, and alcohol, acetic and formic acid in relatively less, proportion than did the original strains from which they were derived, whereas from mannitol there was no diminution in the production of alcohol, acetic, and formic acid.

From these results it is inferred that the artificially selected strains have not lost the enzymes which bring about the final reaction in the production of alcohol and acetic acid, but that the process of artificial selection has led to an absence or diminution of the reducing mechanism of the cell so that some intermediate substance, from which formic acid and the precursor of alcohol and acetic acid are derived, cannot be readily decomposed.

In conclusion I wish to express my thanks to Prof. Harden, F.R.S., in whose laboratory this work has been done.

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On the Genetics of Tetraploid Plants in Primula sinensis.

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The purpose of this paper is to describe certain peculiar results obtained in the genetics of two "giant" races of *Primula sinensis*. Cytological investigation has shown these giants, unlike the giant races already described,* to be in the tetraploid condition, that is to say, that whereas in ordinary Primulas the chromosomes are x (12) in the gametic and $2x$ (24) in the somatic stage, in the tetraploid giants the chromosomes are $2x$ (24) in the gametic and, as nearly as can be counted, $4x$ (48) in the somatic cells.

Nilsson-Ehle† and East‡ have shown that factors of similar property may be reduplicated in the same zygote (or gamete), with various peculiar numerical consequences not otherwise intelligible, notably the appearance in certain F_2-families of such ratios as 15D : 1R, 63D : 1R, and so on, when in the ordinary case 3 : 1 would be expected. The occurrences to be described in part recall this phenomenon; but, as will be seen, they are accompanied by others at first sight entirely paradoxical (as, for example, the fact that the ostensible recessive may throw the dominant), and the whole series may be regarded as of special significance in view of the association with the doubled condition of the cell-constituents. Moreover, in the tetraploid Primulas, the reduplication affects not merely the factors for isolated characters, but extends simultaneously to the factors for all the characters so far investigated.

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* Gregory, 'Camb. Phil. Soc. Proc.,' vol. 15, p. 239 (1909); Keeble, 'Journ. Genetics,' vol. 2, p. 163 (1912).

† "Kreuzungsuntersuchungen an Hafer und Weizen, I and II," 'Lunds Univ. Årsskrift,' 1909 and 1911; 'Berichte d. Deutschen Botanischen Gesellschaft,' vol. 29, p. 65 (1911).

‡ 'American Naturalist,' vol. 44, p. 65 (1910).

The tetraploid giants with which I have worked are of two distinct races. One of these, which will be referred to as the GX race, consists of the progeny of a plant kindly given to me by Messrs. Sutton and Sons. The other race (GT race) arose in the course of my own experiments. Two non-giant diploid plants were crossed together reciprocally. The F_1 from one of these crosses gave a perfectly normal F_2, consisting of non-giant plants among which all the expected classes of offspring were represented in numbers closely approximating to expectation. The F_1 from the reciprocal cross gave no seeds in a cross with one of its parent races and gave only four plants as a result of self-fertilisation. These four plants were giants, and from one of them the GT race has been bred.

Up to the present time, neither the GX nor the GT races of giants have given any fertile seeds in crosses with various non-giant (diploid) races, whichever way the crosses were made. In this respect they differ from a diploid giant race, with which I have worked, which proved quite fertile with non-giants. It was this difference in behaviour which led to the discovery of the tetraploid nature of the GX and GT races.

In the tetraploid plants the chromosomes are naturally more crowded on the spindles than they are in diploid plants, but in polar views of the spindles of either of the maturation divisions there is no difficulty in determining that the number of chromosomes is normally 24 (compared with the 12 chromosomes found in diploid plants). In the somatic mitoses the chromosomes are longer than those of the maturation divisions and exact counts are difficult to make, but a number of counts have given numbers approximating to 48. The maturation divisions sometimes show some degree of irregularity, one or two chromosomes lagging behind the others in the movement to the poles, but I am not yet able to say whether fertile germ cells having more, or fewer, chromosomes than 24 are ever formed. There is no visible difference between the chromosome groups of the thrum-eyed (short-styled) and pin-eyed (long-styled) plants.

The two plants which were the progenitors respectively of the GX and GT races each possessed its own series of dominant characters, in respect of which its origin would indicate that it was heterozygous. In the course of breeding in the direct line from these plants the recessive types have from time to time appeared. The course which this process of throwing recessives has taken is shown in the following table:—

Dominant character of parent.	Recessive character.	Generation in which the recessive first appeared.
	GX Race.	
Petals cut at the edges (*sinensis* type)	Petals heart-shaped with simple median notch (*stellata* variety)	F_3
Dominant white	Magenta flowers	F_4
Green stigma	Red stigma	F_5
Magenta	Red	F_6
Palmate leaves	Fern leaves	F_6
	GT Race.	
Dominant white	Magenta	F_4
Magenta	Red	F_5
Thrum-eyed (short-styled)	Pin-eyed (long-styled)	F_6
Red stems	Green stems	F_6

In the character of the petals and in "dominant white" the dominance of the positive factor is not quite complete and the heterozygous plant can be distinguished from the pure dominant by inspection. In each of these cases, the appearance of the pure recessive is given in the table above, and in each case the heterozygote was recognised in the preceding generation.

In the GT race one expected recessive type, the double flower, has not yet appeared. But in F_3 two plants with semi-double flowers were obtained, both of which would no doubt have produced doubles among their offspring, had they not unfortunately succumbed to the attacks of fungus before they ripened seed.

It is obvious that some of the foregoing recessive characters have made only a belated appearance in the progeny of the original heterozygous plants. Both races of the tetraploid giants, however, produce a relatively very small quantity of seed in self-fertilisation, so that the families raised in each generation have nearly always been small. Consequently, in the present state of our knowledge of the processes of segregation in tetraploid plants, one cannot regard the sporadic appearances of the recessive types as providing a clear indication that processes other than the normal ones are involved.

Besides the recessive types, both races of giants have thrown some peculiar intermediate forms, which are distinct from any intermediate or other forms known to me in the non-giant diploid races. The characters, in respect of which giant intermediates have been produced, include both morphological characters and colour-characters. They are—

	Dominant Character.	Recessive Character.
(1)	Petals cut at the edges.	Petals heart-shaped with simple median notch.
(2)	Tie-ring habit of the inflorescence.	Inflorescence condensed.
(3)	Palmate leaves.	Fern leaves.
(4)	Dominant white.	Coloured flowers.

During the present year there have also been obtained some flower-colours which are intermediate between magenta and red, but, as the diploid races also produce certain colours which it is difficult to classify, further experiment is necessary to show whether or not the new kinds of colour are peculiar to the tetraploid races.

With regard to the characters (1), (2), and (3) above, it should be pointed out that dominance is incomplete in the diploid races, but the giant intermediates form a distinct class from the common heterozygous type, which also occurs in the giant families, alongside of the peculiar intermediate types.

The intermediates between the palmate and fern leaves are, however, the most striking, because in the diploid races the dominance of the palmate shape is, for practical purposes, complete.

In all the cases there is some range of variation among the intermediate forms, and there may be differences of degree between the different organs of the same plant.

Further, it has been found that in the tetraploid giants certain types of flower-coloration may occur, which closely resemble the colours of certain diploid pure races, but are, nevertheless, the product of a different set of factors. This may be simply illustrated in the case of a Giant Red with red stigma, which almost exactly matched the colour of my Red *Stellata* non-giant race. The non-giant race is quite pure, and contains no colour-inhibiting factors. The giant red, selfed, has given (1) forms like itself, (2) more deeply coloured forms, and (3) pure and heterozygous "Duchess" types, that is to say, types showing the possession of the factor which inhibits the production of colour in the peripheral regions of the flower. Other similar cases have occurred, both in plants with green stigmas (*i.e.* possessing the factor which inhibits colour in the central parts of the flower), as well as in those with red stigmas. These cases, then, provide the striking result that the coloured form is shown to be capable of throwing the *dominant* white.

Phenomena of the kind just described, taken together with the fact that the tetraploid giants have produced intermediates peculiar to themselves, suggest some considerations as to the factorial constitution of the tetraploid plants. Both classes of phenomena can, I think, be explained by means of the hypothesis that, as compared with diploid plants, the tetraploid plants possess a double set of factors. Since in the zygote of a diploid pure race each factor is to be regarded as represented twice, AA, it follows that the tetraploid plant, according to this hypothesis, will be AAAA, and the gametes from which such a zygote is formed must be AA, that is to say, the factor will be represented twice in the gamete, instead of once, as it is in the gametes of the ordinary diploid race.

Heterozygous tetraploid plants may, then, be any one of three possible kinds, AAAa, AAaa, Aaaa. Since each gamete will contain two of the four units ("presences" or "absences") which make up the tetraploid group, the gametes produced by the three kinds of heterozygote, and the resulting progeny in F_2, will be as follows:—

Case I.—Heterozygote, AAAa; gametes, AA, Aa;
F_2, 1 AAAA : 2 AAAa : 1 AAaa.

No pure recessives in F_2, but, of every four plants, one will give pure recessives in F_3 in the proportion of one recessive in every 16 plants (see Case 2).

Case II.—Heterozygote, AAaa; gametes, AA, Aa, Aa, aa;
F_2, 1AAAA : 4 AAAa : 6 AAaa : 4 Aaaa : 1 aaaa.

F_2 contains one pure recessive in every 16 plants.

Case III.—Heterozygote, Aaaa; gametes, Aa, aa;
F_2, 1 AAaa ; 2 Aaaa : 1 aaaa.

F_2 contains one pure recessive in every three plants; no pure dominants, but one plant in every four will give pure dominants in F_3.

Of the various kinds of heterozygote shown in the foregoing scheme, one, namely AAaa, has the same proportion of positive and negative elements ("presences" and "absences") as the ordinary diploid heterozygote. With regard to the characters in respect of which the tetraploid giants have produced peculiar intermediates, it is suggested that the intermediates may be either AAAa or Aaaa. The former would presumably show the cumulative effect of the three factors, like that which Nilsson-Ehle and East have recognised in some of their cases, by giving a type more closely resembling the pure dominant than does the ordinary diploid heterozygote, but in the Primulas such types have not yet been definitely recognised by inspection.

Table I.—Crosses of Thrum-eyed and Pin-eyed Plants.

Thrum parent.	F₁.				F₁ plant.	F₁ × self.		F₁ × recessive.		
	Form of cross.	Index No.	Thrum.	Pin.		Thrum.	Pin.	Form of cross.	Thrum.	Pin.
$133^{2}/12$	$133^{2}/12$ as ♂	72/13	6	0	$72^{3}/13$	13	1	$72^{3}/13$ as ♀	3	4
					$72^{5}/13$	14	3	$72^{5}/13$ as ♀	15	19
					$72^{6}/13$	1	0	$72^{6}/13$ as ♀	6	12
	Ditto	86/13	18	1	$86^{2}/13$	7	0	$86^{2}/13$ as ♀	4	1
					$86^{3}/13$	22	1	$86^{3}/13$ as ♀	7	4
								Ditto	1	1
					$86^{4}/13$	19	8	$86^{4}/13$ as ♀	8	2
								$86^{4}/13$ as ♂	9	13
								Ditto	10	9
					$86^{5}/13$	4	4	$86^{5}/13$ as ♀	4	12
								Ditto	11	5
								$86^{5}/13$ as ♂	0	2
								Ditto	7	4
$133^{3}/12$	$133^{3}/12$ as ♂	87/13	15	1	$87^{2}/13$	28	10	$87^{2}/13$ as ♀	22	14
					$87^{3}/13$	27	10	$87^{3}/13$ as ♀	26	22
								$87^{3}/13$ as ♂	1	0
					$87^{5}/13$	8	3	$87^{5}/13$ as ♀	6	12
								Ditto	7	8
					$87^{7}/13$	7	1	$87^{7}/13$ as ♂	5	2
					$87^{9}/13$	30	10	$87^{9}/13$ as ♀	17	16
	Ditto	88/13	21	4	$88^{2}/13$	3	0	$88^{2}/13$ as ♀	7	0
					$88^{3}/13$	4	0			
					$88^{4}/13$	26	11	$88^{4}/13$ as ♀	46	38
								Ditto	3	9
					$88^{6}/13$	37	15	$88^{6}/13$ as ♀	26	25
								Ditto	4	2
					$88^{11}/13$	22	16	$88^{11}/13$ as ♀	5	1
								Ditto	23	18
					$88^{12}/13$	19	9	$88^{12}/13$ as ♀	6	1
								Ditto	14	4
					$88^{13}/13$	7	4	$88^{13}/13$ as ♀	2	0
								Ditto	1	2
								Ditto	1	1
					$88^{18}/13$	2	0	$88^{18}/13$ as ♀	9	9
$133^{5}/12$	$133^{5}/12$ as ♀	68/13	1	0	$68^{1}/13$	44	2	$68^{1}/13$ as ♀	2	1
								Ditto	13	3
								Ditto	1	0
								$68^{1}/13$ as ♂	51	14

The intermediate Aaaa would be expected to show the dominant character in less degree than the normal heterozygote; it is to this class that the intermediates already described are to be assigned.

The intermediate characters do not, however, provide the most favourable opportunity for putting the hypothesis with which we are dealing to a critical statistical test, because the range of variation among the intermediates is sufficient in some families to make classification by inspection a matter of difficulty. This difficulty will no doubt decrease as one becomes more familiar with the new forms, but for the present all that can be said is that the results of the experiments are in general accord with the present hypothesis.

A more critical test is, however, provided by some experiments relating to the characters of thrum-eye and pin-eye, and red stigma and green stigma. In these cases no intermediates have as yet occurred and it may be assumed that one "dose" of the factor is sufficient to bring about the development of the dominant character. The results of these experiments are set out in Tables I and II.

Table II.—Crosses of Green Stigma and Red Stigma.

F_1 plant.	F_1 × self.		F_1 × recessive.		
	Green.	Red.	Form of cross.	Green.	Red.
$72^3/13$	13	0	$72^3/13$ as ♀	5	2
$72^5/13$	17	0	$72^5/13$ as ♀	28	6
$72^6/13$	1	0	$72^6/13$ as ♀	14	4
$68^1/13$	44	2	$68^1/13$ as ♀	15	1
			Ditto	1	0
			Ditto	3	0
			$68^1/13$ as ♂	48	17

In these results two kinds of heterozygous F_1 plants are clearly shown to exist. Thus, in the crosses of thrum × pin (see Table I), the F_1 $68^1/13$ gave 41 thrum 1 pin when selfed, and 65 thrum 15 pin when crossed with the recessive. These numbers may, I think be regarded as representing respectively the ratios 15:1 and 3:1, and the F_1-plant may therefore be identified as AAaa. The thrum parents from which the other F_1's were obtained each gave a small number of recessives in the F_1-families from crosses with recessive plants. It is, therefore, not surprising to find that most of the F_1 thrum plants derived from their crosses have given F_2's approximating to the lower ratios 3:1 and 1:1; that is to say, the majority of the F_1's are of the constitution Aaaa.

To turn to the crosses of green × red stigma (Table II): it should first be pointed out that the GT race sprang from diploid races pure for green stigma and no red stigma has ever appeared in this race bred in the direct line. Plants of this race may, therefore, be written GGGG. It is entirely in accordance with this that the F_1's from crosses of this race with red stigma have all proved to be of the type GGgg, giving the ratios 15 : 1 when selfed, and 3 : 1 when crossed with the recessive. Heterozygotes of the type Gggg have, however, been found by selfing green-stigma plants chosen from families in which some of the plants had red stigmas. Ten such plants have given altogether 99 green stigma, 34 red stigma.

It will be noticed that the F_1's which appear in the green × red-stigma crosses also appear in the thrum × pin crosses. The F_1 $68^1/13$ is giving the same ratios in respect of each pair of characters, namely, 15 : 1 when selfed, and 3 : 1 when crossed by the recessive. But the F_1's 72/13 are giving 15 : 1 and 3 : 1 for green and red stigma, and 3 : 1 and 1 : 1 for thrum and pin. Taking the two pairs of characters together, and assuming for the moment that there are no special inter-relations between the factors, these would give the curious ratios of 45 TG : 15 tG : 3 Tg : 1 tg when the F_1 is selfed, and 3 : 3 : 1 : 1 when the F_1 is crossed by the double recessive (tg). The actual numbers obtained are 28 TG : 4 tG : 0 Tg : 0 tg in the former case, and 22 TG : 25 tG : 2 Tg : 10 tg in the latter.

In the foregoing results the different kinds of heterozygote stand out clearly identified by their progeny, and, although there are considerable discrepancies in individual cases, yet the general trend of these results clearly shows, I think, that the tetraploid plants are endowed with a double set of factors, as compared with the diploid races.

As has been remarked, the results of experiments with the intermediate types are in general accord with the idea that my existing intermediates are heterozygotes of the type Aaaa, in which one "dose" of the factor is not sufficient for the full development of the "dominant" character. The variations among the intermediates themselves, which are probably of the same nature as the variations exhibited among heterozygotes in cases where dominance is imperfect, are, of course, still to be explained. It is curious to notice that when there is any marked variation between the organs of the same plant it appears generally to take the form of a gradual retrogression towards the recessive character in the successively younger and younger organs, the effect of the positive factor being a little less pronounced in each new organ formed.

In conclusion, it must be remarked that the results so far obtained do not of themselves throw any direct light on the problem of the possible relationships between the factors and the chromosomes. Although the fact that the duplication of the chromosomes has been accompanied by a duplication of the series of factors may seem at first sight to suggest a definite connection between chromosomes and factors, yet, on the other hand, the tetraploid number of chromosomes may be nothing more than an index of the quadruple nature of the cell as a whole. The case is, in fact, exactly analogous to the ordinary zygotic cell, which has $2x$ chromosomes and in which each factor is represented twice. But there are grounds for believing that further experiment with tetraploid plants may have a direct bearing in this connection, for some of the experiments have already given an unmistakable indication of the existence of special inter-relations (in the form of coupling or repulsion) between certain factors in the tetraploid Primulas. The work has not yet gone far enough to permit of any useful statement of the results, but it is obvious that it will provide a new opportunity for the study of the mutual relations between factors in heterozygous plants, particularly as to whether or not special inter-relations may occur between the two factors of the same kind (*i.e.* between A and A′), and as to whether either of the factors of one kind may have relations with either factor of another kind (*i.e.* A with either B or B′, and conversely), or whether the A and B factors form one pair of related factors, the A′ and B′ an independent pair, so that A may have special relations with B but none with B′, and conversely.

Part of the expenses of this work have been defrayed by grants from the Royal Society and from the British Association. I wish also to express my great indebtedness to the authorities of the John Innes Horticultural Institution for the facilities for work and the help they have so freely given me.

JUN [illegible] 1914

Description of a Strain of Trypanosoma brucei *from Zululand.*
Part I.—*Morphology.*

By Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S.; Major A. E. Hamerton, D.S.O., and Captain D. P. Watson,* R.A.M.C.; and Lady Bruce, R.R.C. (Scientific Commission of the Royal Society, Nyasaland, 1912–14.)

(Received February 24,—Read March 26, 1914.)

[Plates 21–23.]

Introduction.

In July, 1912, Dr. A. Theiler, C.M.G., Director of Veterinary Research (Union of South Africa), Pretoria, sent this Commission several blood preparations taken from horses and dogs supposed to be suffering from nagana. Much to the surprise of the Commission, a large percentage of these trypanosomes showed posterior-nuclear forms. This disposed of the contention that the so-called *Trypanosoma rhodesiense* could be distinguished from other species of trypanosomes by this peculiarity, and first led the Commission to suspect that *T. rhodesiense* was in reality *T. brucei.*

Dr. Theiler was then asked to send the living strain through to the Commission in Nyasaland, and this, after several failures, was at last successful.

The history of the strain is as follows: Mr. A. W. Shilston, Veterinary Research Division, Pietermaritzburg, writes that it originated in a mule which was naturally infected at Somkele in Zululand. A dog was inoculated from this mule and brought to the Veterinary Research Laboratory at Pietermaritzburg, where sub-inoculations into a series of animals were made. Mr. Shilston says there is no possibility of this strain having been mixed with any other, as, at the time he was working at it, it was the only species of trypanosome maintained at the laboratory. He also states that he—in order to prove that he was dealing with a single species of trypanosome and not with a mixed infection—infected rabbits with single parasites, and the resulting infections showed the same morphological characters as the original strain.

From Pietermaritzburg the strain was transferred to Pretoria. Mr. William Robertson, acting director during the absence on leave of Dr. Theiler, informs the Commission that the strain was kept going in Pretoria in horses and cattle, in which animals it produced the typical clinical symptoms and *post-mortem* lesions associated with nagana, and that it was always regarded as a pure uncomplicated strain of *T. brucei.* The thanks of the Commission are due to Mr. Robertson for his perseverance in sending inoculated animals to their camp at Kasu. Like the Japanese general outside Port Arthur, as one batch succumbed he sent on another, until at last he succeeded.

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* Major Harvey, R.A.M.C., resigned from the Commission and left Kasu, September 16, 1913. He was succeeded by Captain Watson, R.A.M.C., who arrived November 2, 1913.

The exact length of time this trypanosome was kept going at Pretoria before being sent to Kasu is not given, but the information has been asked for, and will be placed on record as soon as obtained.

In the opinion of the Commission, the trypanosome dealt with in this paper is the same as that discovered by Bruce in Zululand in 1894, and named *T. brucei* by Plimmer and Bradford. Somkele is in the same district in Zululand as that in which this species of trypanosome was first discovered.

In this paper the old Zululand strain will be called the 1896 strain, that being the year in which it was first described; the new strain, the 1913 strain, the year in which it was received from Pretoria.

The Zululand trypanosomes were described by Bruce in his original paper* as hæmatozoa which vary among themselves a good deal in size and shape. Photographs were also given, which show a distinct dimorphic type. In a later paper† Bruce gives measurements of 200 trypanosomes taken from preparations which had been made in Zululand in 1896, and also gives six figures taken from the same source. From these it will be seen that the trypanosome dealt with in Zululand in 1896 was a markedly dimorphic form, with long and slender, intermediate, and short and stumpy forms. From the above measurements and figures there cannot be the slightest shadow of doubt about this.

In 1896 Bruce sent this trypanosome to England, and it was at once placed in the hands of Kanthack, Durham, and Blandford by the Royal Society to be reported on. Their investigation lasted two years, and was published in vol. 64 of the 'Proceedings' of the Royal Society. In regard to the shape of this trypanosome they state that "the Nagana parasites vary considerably both in size and form; they may be long and pointed or blunt-ended and somewhat stouter; some individuals are short and thick, with a short flagellum, their protoplasm being crowded with rounded granules." No one who reads Bruce's 'Progress Report' and compares it with Kanthack, Durham, and Blandford's 1898 report can doubt that the same trypanosome was being dealt with. This trypanosome was distinctly dimorphic.

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* 'Further Report on the Tsetse-fly Disease, or Nagana, in Zululand,' 1896.

† 'Roy. Soc. Proc.,' B, vol. 83, p. 9 (1910).

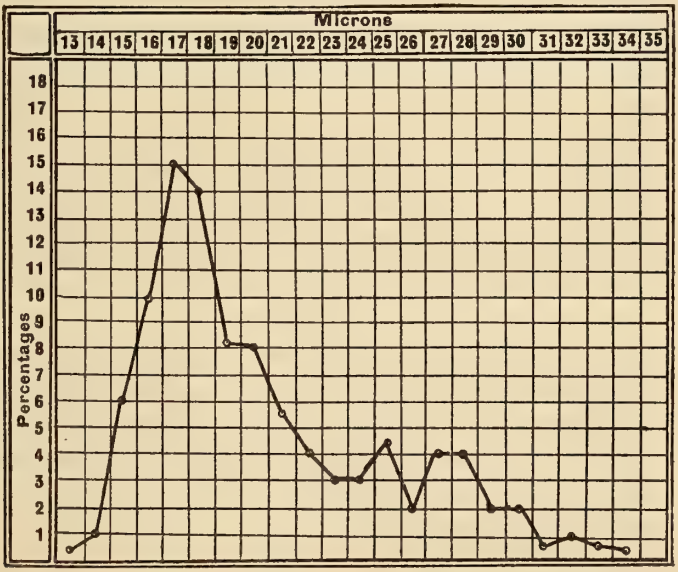
About this time (1898) the trypanosome was handed over to Bradford at the Brown Institute and named *T. brucei* in a paper written in 1899.*

That the trypanosome named by Plimmer and Bradford was the same as that sent to Kanthack, Durham, and Blandford in 1896 there can be no reasonable doubt. There was no other species of pathogenic trypanosome in any English laboratory at the time, with the exception, perhaps, of *T. lewisi*, with which there could be no confusion.

Now, having shown that the original Zululand strain was a well-marked dimorphic type of trypanosome, let us see how it compares with the 1913 strain.

Mr. Shilston kindly sent the Commission a description of this strain made immediately after it had come from Zululand. He states that in the living condition the variation in size and shape of the organism can be observed, the long, slender flagellated forms being readily distinguished from the short, stumpy forms, while all gradations between these two can be found; that the circular vacuole close to the micronucleus is very distinct; and that, although the organisms are actively motile, their progression is not rapid and frequently they simply travel in a small circle.

Chart 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 400 Individuals of *T. brucei*, Zululand Strain, 1913 (Shilston's measurements).



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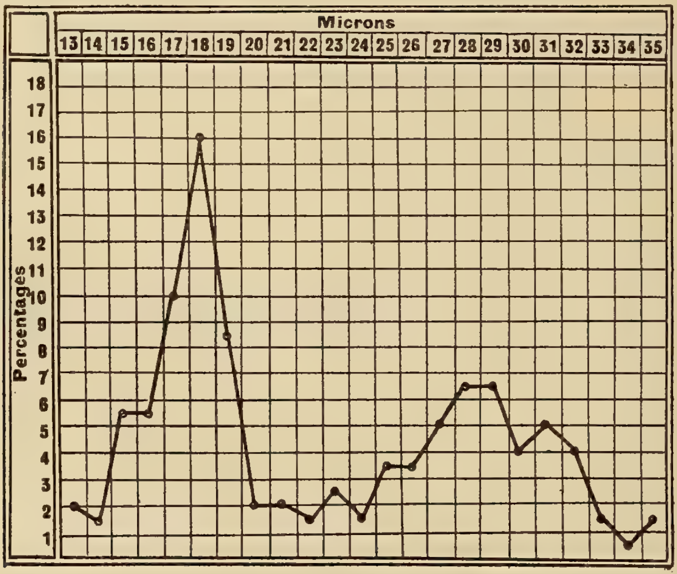
* "A Preliminary Note on the Morphology and Distribution of the Organism found in the Tsetse-fly Disease," by H. G. Plimmer and J. Rose Bradford, 'Roy. Soc. Proc.,' vol. 65, p. 274.

Mr. Shilston also made a large number of measurements of this strain. One of his charts gives the percentages in respect to length of 400 trypanosomes occurring in the mule, dog, and guinea-pig, the measurements being made at varying periods of the disease. This chart is reproduced on p. 495.

In a previous paper* a curve will be found representing the percentages in respect to length of 200 individuals of the original strain of *T. brucei*, measured from old Zululand preparations which had been made in 1896 and were still extant. The numbers making up these two curves are doubtless small, but they are fairly comparable.

CHART 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 200 Individuals of *T. brucei*, Zululand Strain, 1896.



Now although too much weight must not be placed on this comparison, still it must be confessed that the two curves are remarkably alike, and afford a strong argument that Shilston recovered from the Somkele district of Zululand the same species of trypanosome which had been discovered there in 1894. Again, when the action of these two strains on various animals is compared, the same likeness is seen.

Bruce's 1896 strain killed two horses in 30 and 49 days.† Shilston's

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* 'Roy. Soc. Proc.,' B, vol. 83 (1910).

† 'Further Report on the Tsetse-fly Disease, or Nagana, in Zululand,' 1896.

1913 strain killed one horse in 35 days. The former strain killed five dogs in an average of 21 days, the latter four dogs in an average of 19 days.

Taking these various arguments into consideration, it may be assumed that the strain of trypanosome which forms the subject of this paper belongs to the species *T. brucei,* a well-marked dimorphic type of trypanosome.

The object of this paper is to describe as fully as possible the morphology of this new strain of *T. brucei* from Zululand, in order to try to prove its identity with the trypanosome causing disease in man in Northern and Southern Rhodesia, Nyasaland, and German and Portuguese East Africa. The importance of this cannot be overrated. It has been the habit in the past to consider *T. brucei* harmless to man, but if the above conjecture proves to be true, then all *Glossina morsitans* areas where wild game and *T. brucei* co-exist must be looked upon as dangerous. Evidence is accumulating that this is so. Recently two Europeans have fallen victims to the tsetse-fly disease in the Sebungwe district in Southern Rhodesia, a remote, savage, unfrequented spot swarming with game and *G. morsitans.* This year also—1913—as had been anticipated, several cases have been found in the Nyasaland fly-areas to the north and south of the "Proclaimed Area," one case occurring in a native village within a few miles of Zomba, the official capital.

In future papers, the Susceptibility of Animals to this Strain, its Development in *G. morsitans,* Sera Reactions and Cross Inoculation Experiments will be dealt with.

I. MORPHOLOGY OF T. BRUCEI, ZULULAND STRAIN, 1913.

A. *Living, Unstained.*

In the living and unstained preparations the dimorphic characteristics of this species can be readily made out. The parasites are actively motile but with restricted translatory movement.

B. *Fixed and Stained.*

The blood films were fixed, stained and measured as previously described in the 'Proceedings.'*

Length.—The following table gives the length of this trypanosome as found in the monkey, dog, guinea-pig and rat, 1000 trypanosomes in all.

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* B, vol. 81 pp. 16 and 17 (1909).

Table I.—Measurements of the Length of *T. brucei*, Zululand, 1913.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Feb. 10......	1833	Monkey	Osmic acid	Giemsa	21·4	27·0	16·0
,, 10......	1835	,, ...	,,	,,	23·2	29·0	18·0
,, 10......	1836	,, ...	,,	,,	23·4	30·0	18·0
,, 10......	1837	,, ...	,,	,,	21·9	31·0	17·0
,, 13......	1834	,, ...	,,	,,	20·3	28·0	16·0
,, 13......	1835	,, ...	,,	,,	19·0	24·0	12·0
,, 13......	1836	,, ...	,,	,,	20·1	28·0	16·0
,, 17......	1834	,, ...	,,	,,	20·6	27·0	17·0
,, 20......	1904	Dog	,,	,,	22·8	29·0	19·0
,, 20......	1905	,, ...	,,	,,	20·5	29·0	17·0
,, 20......	1907	,, ...	,,	,,	20·6	28·0	17·0
,, 20......	1908	,, ...	,,	,,	20·7	30·0	18·0
,, 24......	1904	,, ...	,,	,,	25·1	31·0	19·0
,, 24......	1905	,, ...	,,	,,	21·1	25·0	18·0
,, 24......	1907	,, ...	,,	,,	20·0	30·0	16·0
,, 24......	1908	,, ...	,,	,,	21·2	25·0	18·0
,, 27......	1904	,, ...	,,	,,	21·7	30·0	18·0
,, 27......	1905	,, ...	,,	,,	21·8	31·0	18·0
,, 27......	1906	,, ...	,,	,,	20·8	28·0	18·0
,, 27......	1907	,, ...	,,	,,	19·1	21·0	16·0
,, 27......	1908	,, ...	,,	,,	24·8	32·0	18·0
,, 24......	1844	Guinea-pig ...	,,	,,	27·6	35·0	18·0
,, 24......	1894	,, ...	,,	,,	21·0	29·0	18·0
,, 27......	1843	,, ...	,,	,,	21·9	29·0	17·0
,, 27......	1894	,, ...	,,	,,	21·2	31·0	17·0
,, 8......	1829	Rat	,,	,,	22·9	28·0	17·0
,, 8......	1829	,, ...	,,	,,	22·5	28·0	18·0
,, 8......	1829	,, ...	,,	,,	21·5	26·0	18·0
,, 9......	1829	,, ...	,,	,,	21·6	25·0	17·0
,, 9......	1829	,, ...	,,	,,	20·9	25·0	18·0
,, 9......	1829	,, ...	,,	,,	20·7	24·0	17·0
,, 10......	1829	,, ...	,,	,,	21·2	24·0	19·0
,, 10......	1829	,, ...	,,	,,	21·6	23·0	19·0
,, 10......	1829	,, ...	,,	,,	21·3	24·0	17·0
,, 11......	1829	,, ...	,,	,,	19·8	25·0	17·0
,, 11......	1829	,, ...	,,	,,	20·5	22·0	18·0
,, 11......	1829	,, ...	,,	,,	20·9	25·0	18·0
,, 12......	1829	,, ...	,,	,,	20·6	23·0	18·0
,, 12......	1829	,, ...	,,	,,	21·7	28·0	18·0
,, 12......	1829	,, ...	,,	,,	21·3	25·0	19·0
,, 13......	1829	,, ...	,,	,,	20·5	23·0	18·0
,, 13......	1829	,, ...	,,	,,	20·0	24·0	17·0
,, 13......	1829	,, ...	,,	,,	20·2	23·0	17·0
,, 14......	1829	,, ...	,,	,,	20·6	26·0	18·0
,, 14......	1829	,, ...	,,	,,	19·5	23·0	17·0
,, 14......	1829	,, ...	,,	,,	20·0	22·0	17·0
,, 15......	1829	,, ...	,,	,,	20·4	23·0	18·0
,, 15......	1829	,, ...	,,	,,	19·7	24·0	17·0
,, 15......	1829	,, ...	,,	,,	20·2	22·0	18·0
,, 16......	1829	,, ...	,,	,,	19·6	22·0	18·0
					21·0	35·0	12·0

The average length of *T. brucei*, Zululand strain, 1913, in the monkey, dog, guinea-pig, and rat, taken from Table I, is as follows :—

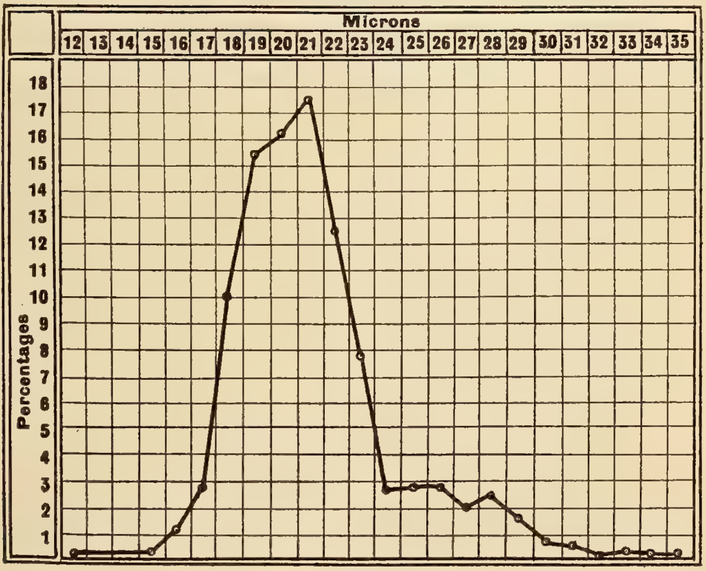
Table II.—Average Length of *T. brucei*, Zululand Strain, 1913.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Monkey	160	21·2	31·0	12·0
Dog	260	21·5	32·0	16·0
Guinea-pig	30	22·9	35·0	17·0
Rat	500	20·8	28·0	17·0

The above table shows that a good deal of difference in growth takes place in different animals. Compare, for example, the guinea-pig with the rat: the former with a maximum of 35 microns, the latter with a maximum of only 28 microns.

The next table gives in detail the distribution in respect to length of 1000 trypanosomes. The Commission feel hardly justified in taking up space for this purpose, but it is thought that perhaps in some unknown way these figures may be of use to the statistician.

Chart 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of *T. brucei*, Zululand Strain, 1913.



This curve is made up of measurements from 160 specimens of trypanosomes taken from the monkey, 260 from the dog, 80 from the guinea-pig, and 500

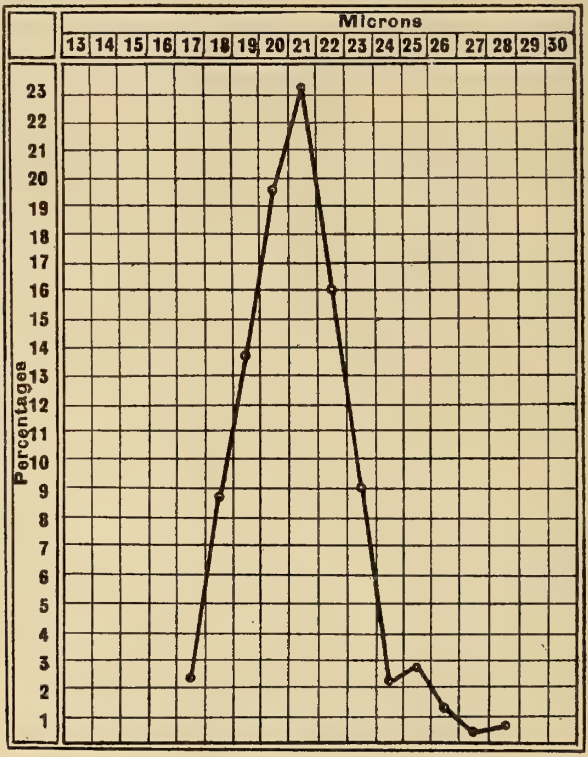
Table III.—Distribution in respect to Length of 500 Individuals of *T. brucei*, Zululand Strain, 1913.

Animal.	In microns.																								Average length.
	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	
Monkey	—	—	—	—	1	—	4	2	1	1	2	4	2	2	—	1	—	—	—	—	—	—	—	—	21·4
„	—	—	—	—	—	—	1	2	2	4	1	2	1	1	1	1	2	2	—	—	—	—	—	—	23·2
„	—	—	—	—	—	—	2	2	1	1	3	—	1	3	5	—	1	—	1	—	—	—	—	—	23·4
„	—	—	—	—	—	1	1	2	3	4	4	1	—	1	1	—	—	1	—	1	—	—	—	—	21·9
„	—	—	—	—	4	—	1	2	1	4	6	1	—	—	—	—	1	—	—	—	—	—	—	—	20·3
„	1	—	—	2	1	1	1	5	3	2	3	—	1	—	—	—	—	—	—	—	—	—	—	—	19·0
„	—	—	—	—	3	1	2	6	2	1	1	—	—	2	—	1	1	—	—	—	—	—	—	—	20·1
„	—	—	—	—	—	2	—	5	4	5	1	—	1	—	1	1	—	—	—	—	—	—	—	—	20·6
Dog	—	—	—	—	—	—	—	2	2	5	1	5	1	—	—	2	—	2	—	—	—	—	—	—	22·8
„	—	—	—	—	—	4	5	3	1	1	1	1	—	—	2	—	1	1	—	—	—	—	—	—	20·5
„	—	—	—	—	—	2	4	—	2	6	3	2	—	—	—	—	1	—	—	—	—	—	—	—	20·6
„	—	—	—	—	—	—	3	3	2	2	—	3	—	—	1	3	2	—	1	—	—	—	—	—	20·7
„	—	—	—	—	—	—	—	3	1	1	1	1	1	—	2	3	2	3	1	1	—	—	—	—	25·1
„	—	—	—	—	—	—	2	2	3	5	4	2	1	1	—	—	—	—	—	—	—	—	—	—	21·1
„	—	—	—	—	1	2	4	2	4	2	3	1	—	—	—	—	—	—	1	—	—	—	—	—	20·0
„	—	—	—	—	—	—	2	4	3	1	2	6	—	2	—	—	—	—	—	—	—	—	—	—	21·2
„	—	—	—	—	—	—	2	4	6	1	1	—	2	—	1	1	1	—	1	—	—	—	—	—	21·7
„	—	—	—	—	—	—	1	6	3	2	4	—	—	—	—	1	2	—	—	1	—	—	—	—	21·8
„	—	—	—	—	—	—	3	6	4	2	—	—	3	—	1	—	1	—	—	—	—	—	—	—	20·8
„	—	—	—	—	1	1	4	6	5	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	19·1
„	—	—	—	—	—	—	2	3	2	—	1	—	—	1	2	1	1	3	2	1	1	—	—	—	24·8
Guinea-pig ...	—	—	—	—	—	—	1	1	—	—	—	2	1	—	1	1	4	2	1	2	—	2	1	1	27·6
„ ...	—	—	—	—	—	—	2	6	5	4	—	—	—	—	—	1	—	2	—	—	—	—	—	—	21·0
„ ...	—	—	—	—	—	1	5	2	1	—	2	3	—	—	4	—	1	1	—	—	—	—	—	—	21·9
„ ...	—	—	—	—	—	1	4	5	2	2	1	—	1	1	—	1	—	—	1	1	—	—	—	—	21·2
Rat	—	—	—	—	—	1	—	1	2	2	3	4	1	2	1	2	1	—	—	—	—	—	—	—	22·9
„	—	—	—	—	—	—	1	2	1	3	4	2	2	2	2	—	1	—	—	—	—	—	—	—	22·5
„	—	—	—	—	—	—	2	1	3	6	3	1	1	2	1	—	—	—	—	—	—	—	—	—	21·5
„	—	—	—	—	—	1	1	2	1	3	4	5	1	2	—	—	—	—	—	—	—	—	—	—	21·6
„	—	—	—	—	—	—	1	3	5	3	6	1	—	1	—	—	—	—	—	—	—	—	—	—	20·9
„	—	—	—	—	—	1	—	3	3	8	4	—	1	—	—	—	—	—	—	—	—	—	—	—	20·7

Rat …………	—	—	—	—	—	—	—	1	6	6	3	2	2	—	—	—	—	—	—	—	—	—	—	—	21·2
" …………	—	—	—	—	—	—	—	1	1	6	8	4	—	—	—	—	—	—	—	—	—	—	—	—	21·6
" …………	—	—	—	—	—	1	1	—	2	4	9	2	1	—	—	—	—	—	—	—	—	—	—	—	21·3
" …………	—	—	—	—	—	1	2	6	5	5	—	—	—	1	—	—	—	—	—	—	—	—	—	—	19·8
" …………	—	—	—	—	—	—	1	3	6	5	5	—	—	—	—	—	—	—	—	—	—	—	—	—	20·5
" …………	—	—	—	—	—	—	1	3	6	5	1	2	—	2	—	—	—	—	—	—	—	—	—	—	20·9
" …………	—	—	—	—	—	—	3	4	2	5	1	5	—	—	—	—	—	—	—	—	—	—	—	—	20·6
" …………	—	—	—	—	—	—	1	2	4	4	3	3	—	1	1	—	1	—	—	—	—	—	—	—	21·7
" …………	—	—	—	—	—	—	—	2	5	4	4	4	—	1	—	—	—	—	—	—	—	—	—	—	21·3
" …………	—	—	—	—	—	—	3	2	2	10	1	2	—	—	—	—	—	—	—	—	—	—	—	—	20·5
" …………	—	—	—	—	—	1	4	4	2	5	2	1	1	—	—	—	—	—	—	—	—	—	—	—	20·0
" …………	—	—	—	—	—	1	3	3	5	2	4	2	—	—	—	—	—	—	—	—	—	—	—	—	20·2
" …………	—	—	—	—	—	—	2	2	6	5	3	1	—	—	1	—	—	—	—	—	—	—	—	—	20·6
" …………	—	—	—	—	—	2	6	2	3	5	1	1	—	—	—	—	—	—	—	—	—	—	—	—	19·5
" …………	—	—	—	—	—	1	1	5	5	6	2	—	—	—	—	—	—	—	—	—	—	—	—	—	20·0
" …………	—	—	—	—	—	—	2	3	8	1	4	2	—	—	—	—	—	—	—	—	—	—	—	—	20·4
" …………	—	—	—	—	—	2	2	5	6	3	—	1	1	—	—	—	—	—	—	—	—	—	—	—	19·7
" …………	—	—	—	—	—	—	2	5	4	5	4	—	—	—	—	—	—	—	—	—	—	—	—	—	20·2
" …………	—	—	—	—	—	—	5	4	5	5	1	—	—	—	—	—	—	—	—	—	—	—	—	—	19·6
Total ………	1	—	—	2	11	28	100	153	161	175	125	79	27	28	28	20	24	17	9	7	1	2	1	1	
Percentages	0·1	—	—	0·2	1·1	2·8	10·0	15·3	16·1	17·5	12·5	7·9	2·7	2·8	2·8	2·0	2·4	1·7	0·9	0·7	0·1	0·2	0·1	0·1	

from the rat. It is very similar to some of the curves taken from the Nyasaland human strain:* compare Strains II and V. But, on the other hand, it is very unlike some of the others, as for example Strains I and II.

Chart 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. brucei*, Zululand Strain, 1913, taken on nine consecutive days from Rat 1829.



This is rather a peculiar curve, but is not unlike curves obtained in a similar way from the trypanosome causing disease in man in Nyasaland, as the following chart will show:—

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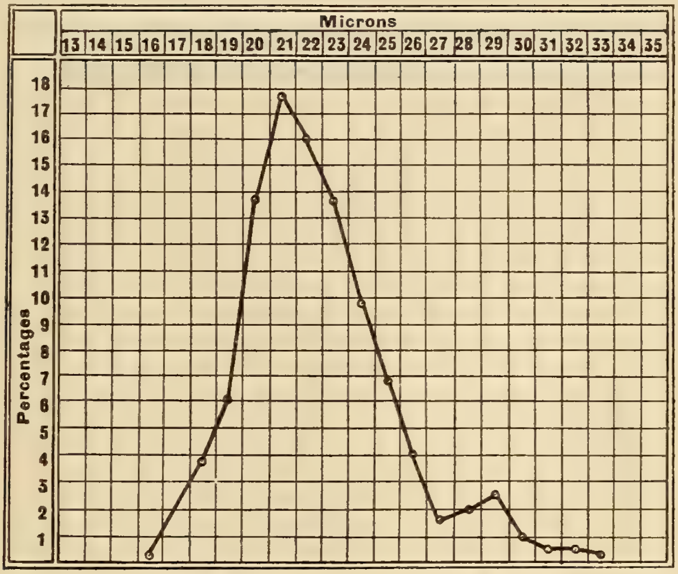
* 'Roy. Soc. Proc.,' B, vol. 86, pp. 285–302.

Chart 5.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome causing Disease in Man in Nyasaland, taken on nine consecutive days from Rat 2300.



The Zululand strain, 1913, has been carried on in horses and cattle for some time in the laboratory at Pretoria, and may have varied somewhat in morphology under these artificial conditions. It will be interesting to see what change, if any, is induced by passage through *G. morsitans.* The three following curves represent first, second and third passages :—

Chart 6.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. brucei*, Zululand Strain, 1913, after first passage through *G. morsitans*, taken on nine consecutive days from Rat 2006.

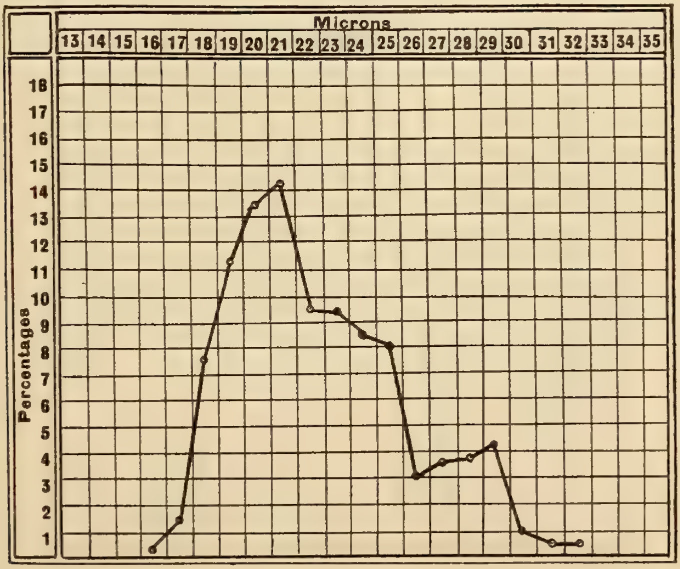


CHART 7.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. brucei*, Zululand Strain, 1913, after second passage through *G. morsitans*, taken on nine consecutive days from Rat 2288.

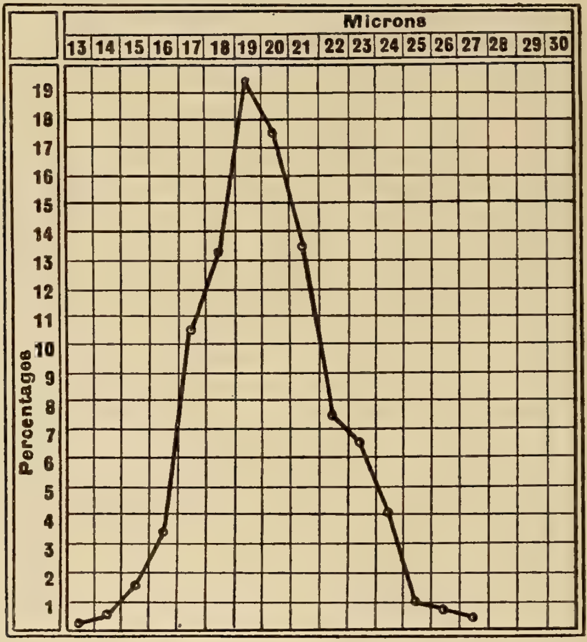
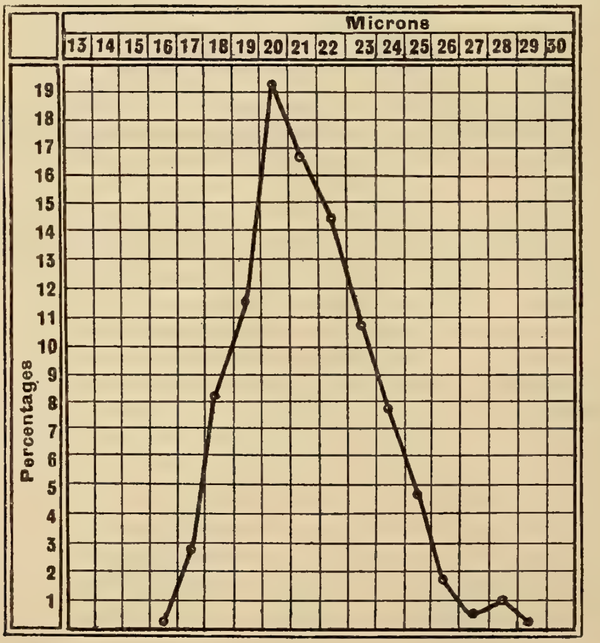


CHART 8.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of *T. brucei*, Zululand Strain, 1913, after third passage through *G. morsitans*, taken on nine consecutive days from Rat 2406.



From this curve it will be seen that three passages through *G. morsitans* has had little or no effect in changing the character of this trypanosome as regards distribution of length. It has usually been thought that a trypanosome kept under laboratory conditions, and without the opportunity of passage through its invertebrate host, the tsetse fly, would tend to change in morphology. These curves, on the other hand, show that a trypanosome, after passage through horses and cattle for some years—exact time unknown—is unchanged by three passages through its invertebrate host, *G. morsitans.*

Table IV.—Distribution in respect to Length of 500 Individuals of *T. brucei,* Zululand Strain, 1913, after first passage through *G. morsitans.*

Animal.	In microns.																	Average length.
	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	
Rat	—	1	1	5	—	2	1	1	4	1	—	2	—	2	—	—	—	22·5
,, ...	—	—	—	1	3	—	4	3	1	3	—	1	3	—	1	—	—	23·8
,, ...	—	1	3	—	1	4	2	4	1	1	1	—	1	1	—	—	—	22·1
,, ...	—	—	3	—	5	2	3	3	1	2	—	—	—	1	—	—	—	21·7
,, ...	—	1	1	1	2	6	3	1	4	—	—	—	1	—	—	—	—	21·4
,, ...	—	—	2	—	3	6	4	2	2	1	—	—	—	—	—	—	—	21·4
,, ...	—	—	—	3	4	4	2	2	1	1	—	1	1	—	—	—	1	22·3
,, ...	—	—	3	1	8	3	—	1	1	2	—	1	—	—	—	—	—	21·0
,, ...	—	—	3	3	5	1	2	2	—	—	—	2	—	2	—	—	—	21·7
,, ...	—	—	2	2	1	—	1	2	2	1	1	—	2	4	2	—	—	24·6
,, ...	—	—	—	3	3	1	1	—	2	4	1	—	1	2	—	1	1	24·1
,, ...	—	—	1	2	—	1	—	3	4	4	1	1	1	2	—	—	—	24·0
,, ...	—	—	2	4	1	1	4	—	2	3	—	2	—	—	1	—	—	22·4
,, ...	—	—	1	1	1	1	2	2	5	3	2	—	—	1	—	1	—	23·7
,, ...	—	—	1	1	1	2	2	3	3	3	2	1	—	1	—	—	—	23·3
,, ...	—	2	2	2	1	3	1	3	2	1	—	2	—	1	—	—	—	21·9
,, ...	—	1	3	1	2	3	4	—	—	4	—	—	2	—	—	—	—	21·8
,, ...	—	—	2	3	2	5	1	5	—	1	—	1	—	—	—	—	—	21·3
,, ...	—	—	1	5	4	5	1	—	—	—	2	1	—	1	—	—	—	21·4
,, ...	1	—	1	1	3	3	2	4	1	1	—	—	2	1	—	—	—	22·3
,, ...	—	—	1	5	1	4	2	1	3	—	1	—	1	1	—	—	—	21·9
,, ...	—	—	1	4	5	4	1	2	—	1	—	1	1	—	—	—	—	21·3
,, ...	—	—	1	2	3	5	2	2	1	1	1	1	—	1	—	—	—	22·1
,, ...	—	—	—	1	4	4	3	1	2	2	1	—	1	—	1	—	—	22·7
,, ...	—	1	3	5	4	1	—	—	1	—	2	1	2	—	—	—	—	21·3
Total...........	1	7	38	56	67	71	48	47	43	40	15	18	19	21	5	2	2	
Percentages ...	0·2	1·4	7·6	11·2	13·4	14·2	9·6	9·4	8·6	8·0	3·0	3·6	3·8	4·2	1·0	0·4	0·4	

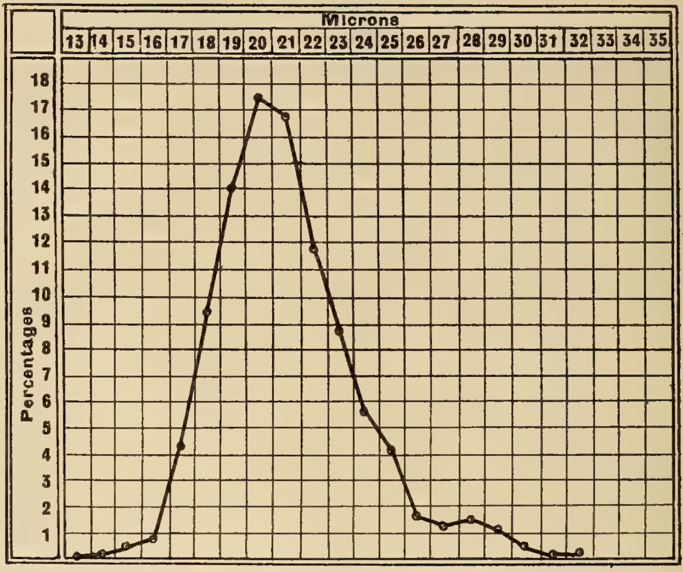
Table V.—Distribution in respect to Length of 500 Individuals of *T. brucei*, Zululand Strain, 1913, after second passage through *G. morsitans*.

Animal.	In microns.															Average length.
	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	
Rat	—	—	—	—	1	2	—	5	4	2	3	2	1	—	—	21·1
,, ...	—	—	—	—	2	—	4	3	3	1	4	2	—	1	—	21·0
,, ...	—	—	—	—	1	2	2	3	2	1	2	4	1	2	—	21·7
,, ...	—	—	—	—	1	3	4	5	4	2	1	—	—	—	—	19·9
,, ...	—	—	—	—	2	2	9	3	3	1	—	—	—	—	—	19·3
,, ...	—	—	1	—	3	2	5	5	4	—	—	—	—	—	—	19·0
,, ...	—	—	1	3	4	—	5	1	2	1	1	1	1	—	—	19·1
,, ...	—	—	1	—	5	2	2	4	4	1	—	—	—	—	1	19·3
,, ...	—	—	—	2	—	2	5	3	2	4	2	—	—	—	—	19·9
,, ...	—	—	—	2	1	3	3	5	3	—	2	1	—	—	—	19·6
,, ...	—	—	1	—	2	3	5	5	3	—	—	—	—	—	1	19·4
,, ...	—	—	—	1	3	4	1	3	4	2	1	—	—	1	—	19·7
,, ...	—	2	1	3	4	3	2	2	2	1	—	—	—	—	—	17·7
,, ...	1	—	—	1	2	4	6	3	—	—	3	—	—	—	—	18·9
,, ...	—	—	1	1	6	5	3	3	—	—	1	—	—	—	—	18·1
,, ...	—	—	—	2	3	5	2	3	1	2	1	1	—	—	—	19·1
,, ...	—	—	—	—	2	1	7	5	4	1	—	—	—	—	—	19·5
,, ...	—	—	—	—	4	3	4	4	3	—	1	1	—	—	—	19·4
,, ...	—	—	1	1	3	3	3	2	5	1	1	—	—	—	—	19·1
,, ...	—	—	1	—	—	2	5	5	—	6	1	—	—	—	—	20·0
,, ...	—	—	—	—	—	1	7	5	3	1	1	2	—	—	—	20·3
,, ...	—	—	—	—	1	2	4	5	2	2	2	2	—	—	—	20·4
,, ...	—	—	—	—	2	2	2	1	4	5	3	—	1	—	—	20·8
,, ...	—	—	—	—	1	3	2	2	3	3	1	4	1	—	—	21·1
,, ...	—	—	—	1	—	7	5	3	2	—	2	—	—	—	—	19·2
Total	1	2	8	17	53	66	97	88	67	37	33	20	5	4	2	
Percentages ...	0·2	0·4	1·6	3·4	10·6	13·2	19·4	17·6	13·4	7·4	6·6	4·0	1·0	0·8	0·4	

Table VI.—Distribution in respect to Length of 500 Individuals of *T. brucei*, Zululand Strain, 1913, after third passage through *G. morsitans*.

Animal.	In microns.														Average length.
	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	
Rat	—	—	1	2	3	2	5	4	2	1	—	—	—	—	21 ·6
,, ...	—	—	3	1	3	5	3	—	2	3	—	—	—	—	21 ·3
,, ...	—	1	1	—	3	6	3	4	—	2	—	—	—	—	21 ·4
,, ...	—	—	2	1	2	6	1	4	2	—	1	—	1	—	21 ·8
,, ...	—	—	1	1	1	3	3	5	4	2	—	—	—	—	22 ·3
,, ...	—	—	2	2	5	2	2	3	2	—	1	1	—	—	21 ·5
,, ...	—	—	—	3	5	8	2	1	1	—	—	—	—	—	20 ·8
,, ...	—	1	1	4	5	2	3	2	1	1	—	—	—	—	20 ·7
,, ...	—	1	1	3	2	7	3	1	1	—	—	—	1	—	21 ·0
,, ...	—	2	—	3	4	1	5	4	1	—	—	—	—	—	20 ·8
,, ...	—	—	1	3	6	4	2	2	2	—	—	—	—	—	20 ·8
,, ...	—	—	1	1	3	4	2	5	2	1	1	—	—	—	21 ·9
,, ...	—	—	1	4	2	1	1	2	3	5	1	—	—	—	22 ·3
,, ...	1	—	3	3	3	—	4	3	—	1	1	—	1	—	21 ·1
,, ...	—	—	1	2	2	1	8	1	3	1	—	—	—	1	22 ·1
,, ...	—	2	1	1	7	3	2	2	1	1	—	—	—	—	20 ·6
,, ...	—	—	2	2	4	6	3	1	—	1	—	1	—	—	21 ·0
,, ...	—	—	3	3	8	1	1	—	2	—	1	—	1	—	20 ·8
,, ...	—	1	1	5	4	1	2	1	2	2	1	—	—	—	21 ·1
,, ...	—	1	3	3	2	4	3	3	1	—	—	—	—	—	20 ·5
,, ...	—	1	4	3	5	2	1	2	—	2	—	—	—	—	20 ·3
,, ...	—	—	3	1	5	4	4	—	2	—	—	—	1	—	21 ·0
,, ...	—	2	1	1	5	2	4	2	1	1	1	—	—	—	21 ·1
,, ...	—	2	3	3	1	4	4	1	2	—	—	—	—	—	20 ·4
,, ...	—	—	1	3	6	5	1	1	2	—	1	—	—	—	20 ·9
Total	1	14	41	58	96	84	72	54	39	24	9	2	5	1	
Percentages	0 ·2	2 ·8	8 ·2	11 ·6	19 ·2	16 ·8	14 ·4	10 ·8	7 ·8	4 ·8	1 ·8	0 ·4	1 ·0	0 ·2	

Chart 9.—Curve representing the Distribution, by Percentages, in respect to Length, of 2000 Individuals of *T. brucei,* Zululand Strain, 1913, taken on nine consecutive days from Rats 1829, 2006, 2288, and 2406.



As Shilston's mule was in all probability infected from the wild game of the Somkele district, in Zululand, it will be interesting to compare this curve with that of the trypanosome causing disease in man, taken from the wild game in Nyasaland.

Chart 10.—Curve representing the Distribution, by Percentages, in respect to Length, of 2500 Individuals of the Trypanosome causing Disease in Man in Nyasaland, the Wild-game Strain, taken on nine consecutive days from Rats 847, 1220, 992, 849, and 1022.

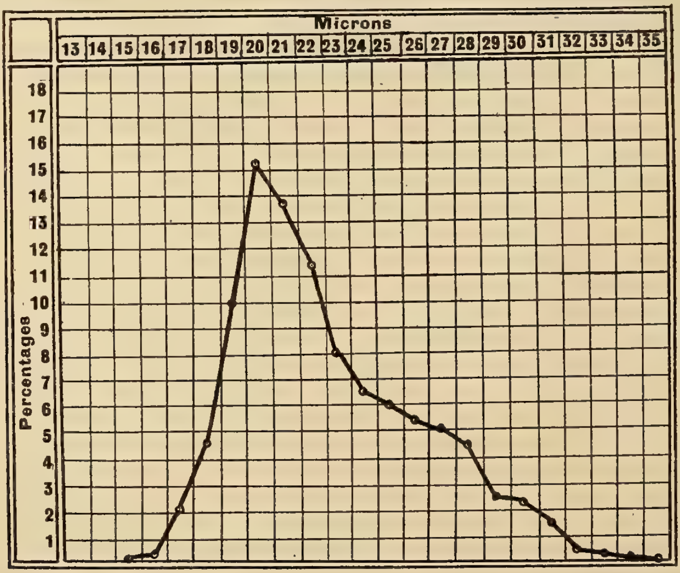


Table VII.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of *T. brucei*, Zululand Strain, 1913.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
June 22	I	Horse	30
,, 25	II	,,	65
—	I	Dog	5
June 21	II	,,	Nil
1913.			
Feb. 8	1828	Rat	1
,, 9	1828	,,	37
,, 10	1828	,,	63
,, 11	1828	,,	36
,, 12	1828	,,	32
,, 13	1828	,,	17
,, 14	1828	,,	54
,, 15	1828	,,	45
,, 16	1828	,,	20
,, 17	1828	,,	17
Mar. 24	2006	,,	10
,, 25	2006	,,	54
,, 26	2006	,,	41
,, 27	2006	,,	18
,, 28	2006	,,	38
,, 29	2006	,,	32
Apr. 1	2006	,,	69
Mar. 2	2006	,,	74
,, 3	2006	,,	63
,, 7	2006	,,	74
July 21	2288	,,	Nil
,, 22	2288	,,	42
,, 23	2288	,,	20
,, 24	2288	,,	35
,, 25	2288	,,	16
,, 26	2288	,,	29
,, 27	2288	,,	43
,, 28	2288	,,	52
,, 29	2288	,,	49
,, 30	2288	,,	61
—	I	Mouse	5
		Average	37·8

These two curves are undoubtedly much alike, and as the wild-game strain in Nyasaland is supposed to be identical with the human strain, then it might be said that *T. brucei*, Zululand strain, 1913, is also identical with the trypanosome causing disease in man in Nyasaland. Others will say that the Zululand trypanosome and the Nyasaland wild-game strain are both *T. brucei*, but that this does not prove that *T. brucei* is identical with the human strain. No, but if it is shown that *T. brucei*, Zululand, is absolutely identical in morphology with this Nyasaland human strain, that it also has exactly the same disease-producing power on the various experimental animals, this will go a good long way to make the ordinary unprejudiced man chary of exposing himself too carelessly to the so-called harmless nagana. His serum may save him as a rule, but there may come a time when—his resistance being lowered either by fatigue, or some other cause—the trypanosome may gain a footing, and then his belief in the written word of the text-book will receive a rude shock.

Breadth.—The long and slender average 2·76 microns in breadth, the intermediate 3·25, and the short and stumpy 3·53. This measurement was made across the broadest part of the trypanosome and includes the undulating membrane. Most previous measurements of breadth have not included this.

In regard to the shape of this trypanosome, contents of cell, nucleus micronucleus, undulating membrane, and flagellum, it is not proposed to describe these characters separately for this strain as was done in the case of the trypanosome causing disease in man in Nyasaland. Suffice it to say that, after the most careful comparison, no difference whatever can be made out in the morphology of the two trypanosomes. At the end of this paper three plates are given, one representing the short and stumpy, another the intermediate, and a third the long and slender forms. If these plates are compared with those given in previous papers* it will be seen that in morphology the Nyasaland and Zululand trypanosomes are identical.

CONCLUSIONS.

1. The trypanosome described in this paper under the name of the "Zululand strain, 1913," is the same species as that discovered by Bruce in Zululand in 1894; reported on by Kanthack, Durham and Blandford in 1898; and named *T. brucei* by Plimmer and Bradford in 1899.

2. As regards its morphology, this trypanosome is absolutely identical with the trypanosome causing disease in man in Nyasaland, the *T. rhodesiense* of Stephens and Fantham.

DESCRIPTION OF PLATES.

PLATE 21.

Trypanosoma brucei, Zululand strain, 1913, short and stumpy forms. × 2000.

PLATE 22.

Trypanosoma brucei, Zululand strain, 1913, intermediate forms. × 2000.

PLATE 23.

Trypanosoma brucei, Zululand strain, 1913, long and slender forms. × 2000.

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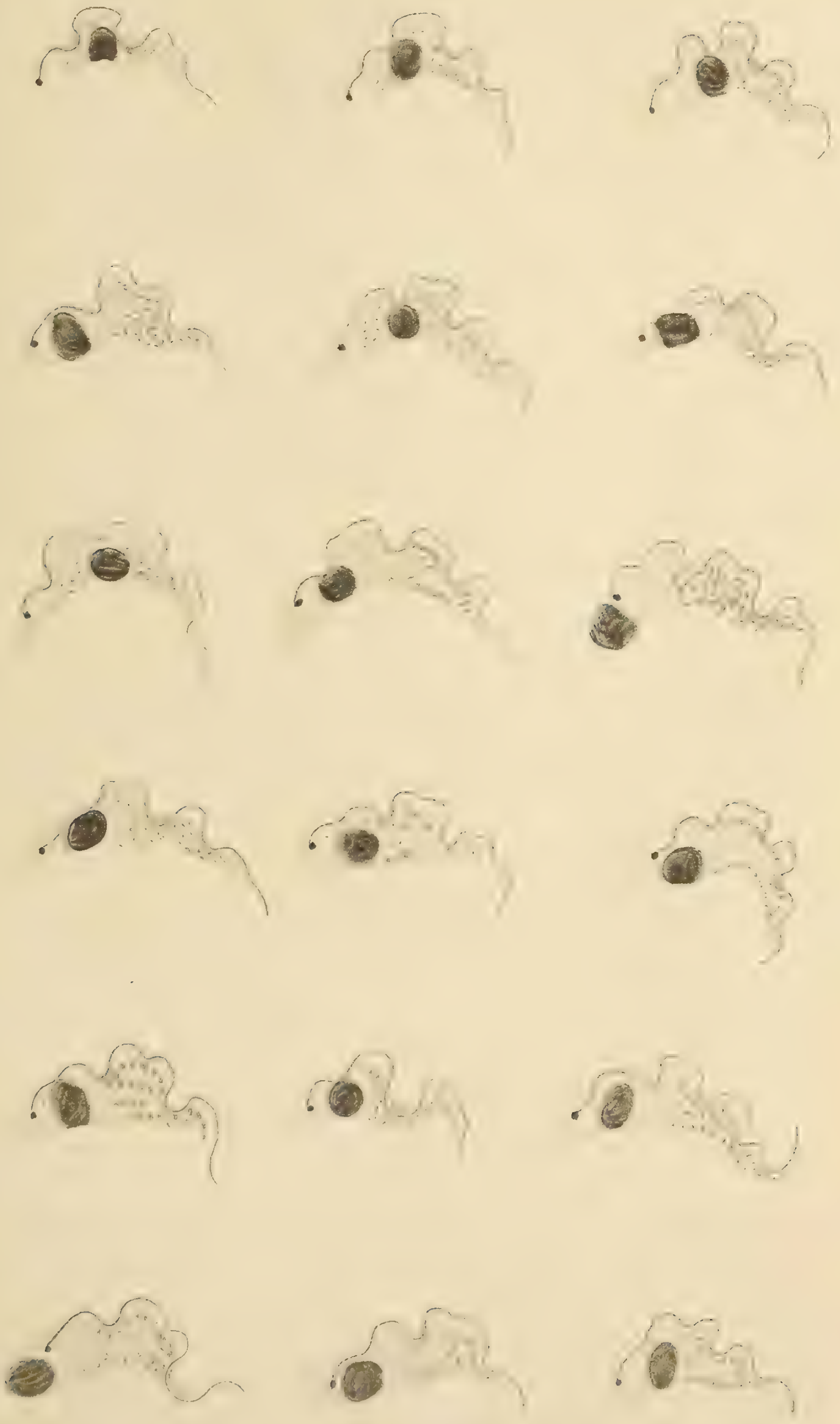
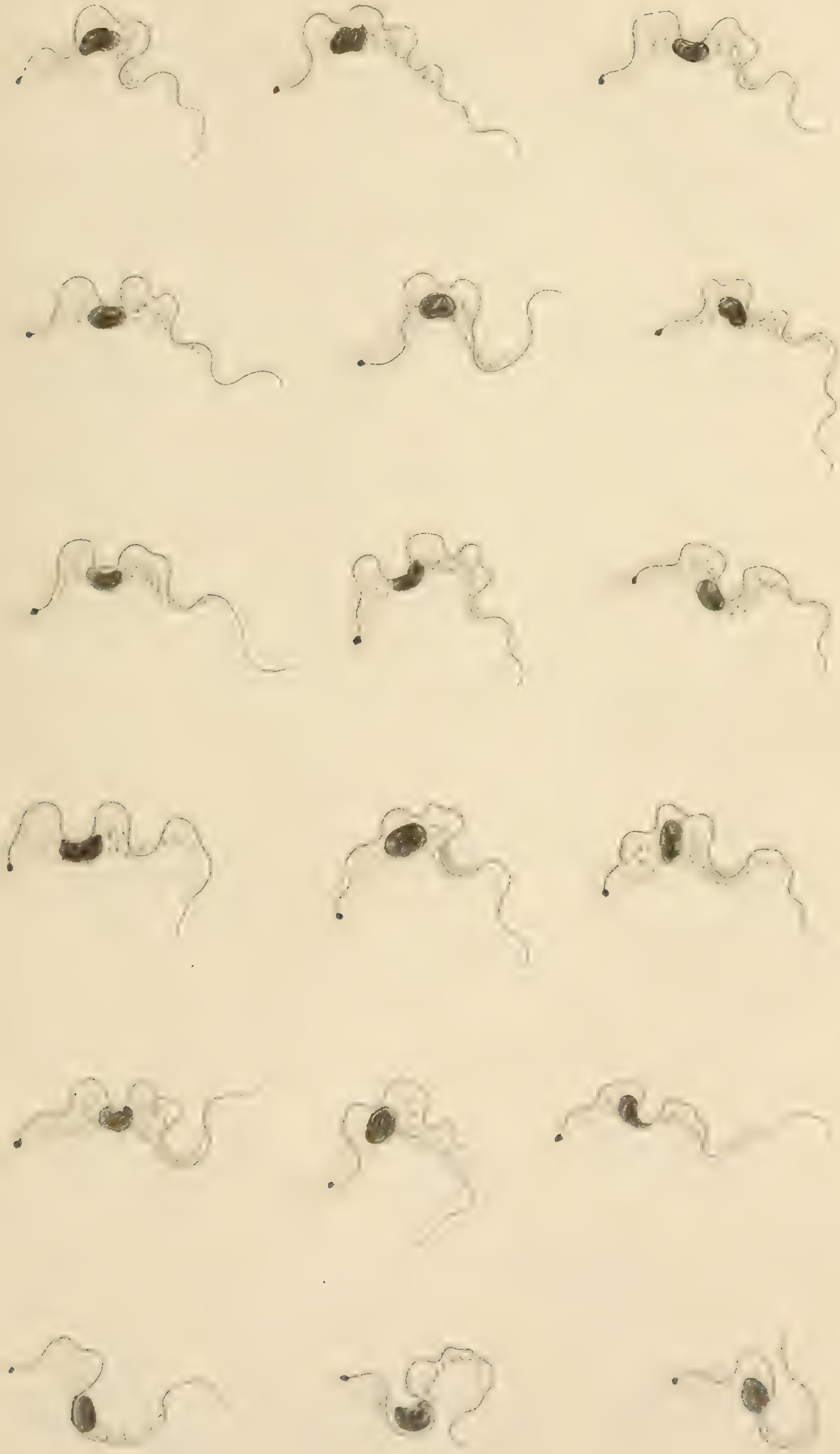
* 'Roy. Soc. Proc.,' B, vol. 85, p. 433 (1912), and vol. 87, p. 35 (1913).



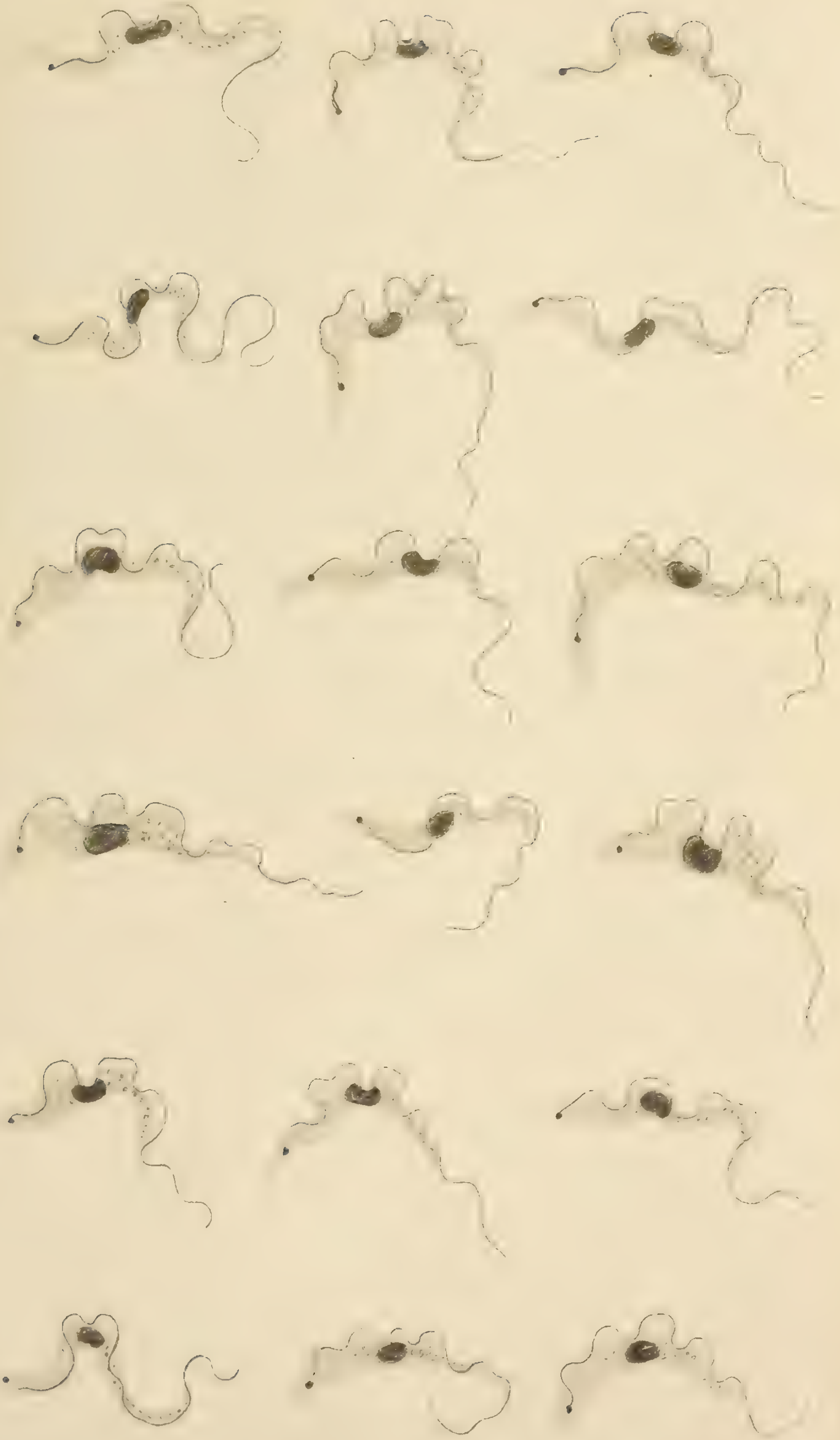
Short & Stumpy.

M. E. Bruce del.



Intermediate.

M. E. Bruce, del.



Long & Slender.

M. E. Bruce, del.

Description of a Strain of Trypanosoma brucei *from Zululand.*
Part II.—*Susceptibility of Animals.*

By Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S.; Major A. E. Hamerton, D.S.O., and Captain D. P. Watson, R.A.M.C.; and Lady Bruce, R.R.C. (Scientific Commission of the Royal Society, Nyasaland, 1912–14.)

(Received February 24,—Read March 26, 1914.)

Introduction.

In the foregoing paper the morphology of this trypanosome was described, and the conclusion arrived at that it is identical, as regards shape, size and general appearance, with the trypanosome causing disease in man in Nyasaland, the *Trypanosoma rhodesiense* of Stephens and Fantham.

This paper describes the action on animals of the Zululand trypanosome, and it is compared in this regard with the Nyasaland species.

Susceptibility of Animals to T. brucei, Zululand Strain, 1913.

Table I.

Date.	No. of expt.	Source of virus.	Incubation, in days.	Duration, in days.*	Remarks.
			Horse.		
1895.					
—	37	Dog 4	3	35	"Very old animal; typical Nagana."—Shilston.
Sept. 27	212	Natural infection	?	30	Zululand, 1896, Bruce.
" 29	235	" "	6	49	" " "
		Average	—	38·0	
			Ox.		
1913.					
—	22	Dog 4	6	—	"Still alive after 90 days."—Shilston.
Feb. 18	1913	Rat 1839	—	—	Never showed trypanosomes.
" 18	1914	" 1839	37	—	Still alive after 316 days.
" 18	1915	" 1839	35	310	Died of *T. brucei*.
July 22	2304	Dog 2281	—	—	Never showed trypanosomes.
" 22	2305	" 2281	—	—	" "
" 22	2306	" 2281	—	—	" "
			Sheep.		
—	1	Dog 4	12	—	"Still alive after 90 days."—Shilston.

* Duration includes the days of incubation; it dates from day of infection.

Table I—*continued.*

Date.	No. of expt.	Source of virus.	Incubation, in days.	Duration, in days.*	Remarks.
		Goat.			
Feb. 12...	1887	Rats 1832 and 1838 ...	—	—	Never showed trypanosomes.
,, 12...	1888	,, 1832 and 1838 ...	—	—	,, ,,
,, 12...	1889	,, 1832 and 1838 ...	15	109	Died of *T. brucei.*
,, 12...	1891	,, 1832 and 1838 ...	—	—	Never showed trypanosomes.
Mar. 15...	1890	Guinea-pigs 1840 and 1843	12	45	Died of *T. brucei.*
July 16...	2290	Dog 2254..................	26	39	,, ,,
,, 16...	2291	,, 2254..................	36	116	,, ,,
		Average......	22·2	77·2	
		Monkey.			
Feb. 3...	1833	Rabbit 1830	7	8	Died of *T. brucei.*
,, 3...	1834	,, 1830	7	15	,, ,,
,, 3...	1835	,, 1830	7	14	,, ,,
,, 3...	1836	,, 1830	7	49	,, ,,
,, 3...	1837	,, 1830	7	16	,, ,,
,, 17...	1970	Laboratory-bred flies...	—	17	,, ,,
July 16...	2292	Dog 2254..................	5	50	,, ,,
,, 16...	2293	,, 2254..................	5	65	,, ,,
		Average......	6·4	29·2	
		Dog.			
Feb. 14...	1904	Monkey 1835	6	26	Died of *T. brucei.*
,, 14...	1905	,, 1835	6	17	,, ,,
,, 14...	1906	,, 1835	10	18	,, ,,
,, 14...	1907	,, 1835	6	23	,, ,,
,, 14...	1908	,, 1835	6	21	,, ,,
April 1...	2047	Rat 2027..................	6	15	,, ,,
,, 1...	2048	,, 2027..................	6	20	,, ,,
,, 1...	2049	,, 2027..................	6	14	,, ,,
,, 1...	2050	,, 2027..................	6	17	,, ,,
,, 1...	2051	,, 2027..................	6	20	,, ,,
,, 22...	2104	,, 2065..................	6	17	,, ,,
June 24...	2240	Guinea-pig 2225.........	7	12	,, ,,
July 3...	2254	Laboratory-bred flies...	?	17	,, ,,
,, 9...	2281	Guinea-pig 2225	5	20	,, ,,
,, 16...	2294	Dog 2254..................	5	19	,, ,,
,, 16...	2295	,, 2254..................	5	22	,, ,,
Aug. 11...	2361	Wild flies	?	16	,, ,,
		Average......	6·1	18·5	
		Rabbit.			
—	145	Dog 1	—	33	Shilston.
—	146	,, 1	—	39	,,
—	150	,, 2	—	31	,,
—	157	Rat 4A.....................	13	34	,,
—	158	Horse 37..................	3	27	,,
—	165	Rabbit 158	5	27	,,
Jan. 13...	1897	Pretoria strain	?	36	Died of *T. brucei.*
,, 17...	1830	,, ,, ...	?	35	,, ,,
		Average......	7·0	32·7	

* Duration includes the days of incubation; it dates from day of infection.

Table I—*continued.*

Date.	No. of expt.	Source of virus.	Incubation, in days.	Duration in days.*	Remarks.
			Guinea-pig.		
Feb. 3...	1840	Rabbit 1830	31	50	Died of *T. brucei.*
„ 3...	1841	„ 1830	35	50	„ „
„ 3...	1842	„ 1830	—	—	Never showed trypanosomes.
„ 3...	1843	„ 1830	21	59	Died of *T. brucei.*
„ 3...	1844	„ 1830	14	37	„ „
„ 13...	1895	Pretoria strain	—	—	Never showed trypanosomes.
„ 13...	1896	„ „	—	—	„ „
Mar. 28...	1842	Monkey 1970	10	27	Reinjected; died of *T. brucei.*
„ 28...	1895	„ 1970	20	46	„ „ „
„ 28...	1896	„ 1970	20	30	„ „ „
May 13...	2136	„ 1970	—	—	Never showed trypanosomes.
„ 29...	2136	„ 1970	4	15	Reinjected; died of *T. brucei.*
June 13...	2225	„ 1970	3	34	Died of *T. brucei.*
July 16...	2296	Dog 2254....................	47	89	„ „
		Average......	20·5	43·7	
			Rat.		
Feb. 2...	1828	Rabbit 1830	8	19	Died of *T. brucei.*
„ 2...	1829	„ 1830	6	58	„ „
„ 3...	1832	„ 1830	7	46	„ „
„ 3...	1838	„ 1830	10	13	„ „
„ 3...	1839	„ 1830	7	22	„ „
„ 14...	1902	„ 1897	17	31	„ „
„ 26...	1966	Guinea-pig 1844	8	23	„ „
Mar. 15...	1993	Rat 1832....................	5	13	„ „
„ 15...	1994	„ 1832....................	5	10	„ „
„ 15...	1995	„ 1832....................	5	30	„ „
„ 19...	2006	Monkey 1970	5	24	„ „
April 4...	2065	„ 1836	7	30	„ „
„ 11...	2073	Rat 2065....................	4	17	„ „
May 13...	2135	Monkey 1970	6	33	„ „
„ 13...	2137	Goat 1889	6	18	„ „
„ 13...	2138	„ 1889	6	17	„ „
„ 29...	2196	Rat 2135....................	11	26	„ „
July 16...	2288	Dog 2254....................	5	33	„ „
„ 16...	2289	„ 2254....................	5	44	„ „
Sept. 2...	2406	„ 2361....................	6	24	„ „
„ 16...	2412	Rat 2406....................	6	34	„ „
Oct. 20...	2423	„ 2412....................	8	39	„ „
Nov. 28...	2442	„ 2431....................	4	16	„ „
		Average......	6·8	27·0	

* Duration includes the days of incubation; it dates from day of infection.

Disease set up in Various Animals by T. brucei, *Zululand Strain,* 1913.

Horse.—The Commission had no opportunity of studying this strain in the horse, but Mr. Shilston states that one horse inoculated by him at Pietermaritzburg died in 35 days with typical symptoms of Nagana.

Ox.—Six oxen were inoculated, but only two of these at any time showed trypanosomes in their blood. One of these died after 310 days, while the other is still alive at the end of a year. This animal has evidently recovered, as it appears sleek and healthy. The action of the Zululand strain is therefore the same as that of the trypanosome causing disease in man in Nyasaland: neither of them show any marked power of producing serious disease in cattle.

Goat.—Seven goats were inoculated with this strain. Four died, on an average, in 77·2 days (45 to 116). The remaining three proved refractory. No œdema of face or corneal opacity was noted in any of the goats. The Zululand strain seems to have less action on goats than the Nyasaland trypanosome, but the number of experiments is small. In the latter the duration of the disease was 41·8 days (19 to 72).

Sheep.—No experiments were made with these animals in Nyasaland as it was found impossible to procure them from the natives.

Monkey.—Eight monkeys died, on an average, in 29·2 days (8 to 65). The trypanosomes were always present in the blood, sometimes in enormous numbers. In no case was œdema of the face or corneal opacity noted. After death, enlargement of the spleen and liver, gelatinous infiltration at the base of the heart, and hæmorrhages in the epicardium were found.

Dog.—Seventeen dogs were inoculated. All died, on an average, in 18·5 days (12 to 26). In eight dogs blindness caused by opacity of the cornea was a prominent symptom, and in two swellings of the limbs were observed.

Rabbit.—As only two rabbits were available at Kasu, six experiments reported by Mr. Shilston are added. Eight rabbits died, on an average, in 32·7 days (27 to 39). The course of the disease in the Kasu rabbits was the same as that described in a former paper* as being typical of Nagana.

Guinea-pig.—This animal is less affected by the disease than the rabbit. Ten were used; all took the disease and died, but four required to be inoculated more than once. They died, on an average, in 43·7 days (15 to 89). No prominent symptoms, such as are seen in the rabbit, occur in the guinea-pig.

Rat.—Twenty-three were inoculated and died, on an average, in 27 days (10 to 58), with their blood swarming with trypanosomes and their spleens enormously enlarged.

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* "The Trypanosome causing Disease in Man in Nyasaland.—Susceptibility of Animals o the Human Strain," 'Roy. Soc. Proc.,' B, vol. 87 (1913).

Table II.—The Average Duration, in Days, of the Disease in Various Animals caused by *T. brucei,* Zululand Strain, 1913.

	Horse.	Ox.	Goat.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Average duration, in days	38	310	77	29	18	33	44	27
Number of animals employed	3	1	7	8	17	8	10	23

Compare this with the following table:—

Table III.—The Average Duration of Life, in Days, of Various Animals Infected with the Human Strain of the Trypanosome causing Disease in Man in Nyasaland.

	Horse.	Ox.	Goat and sheep.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Average duration, in days	0	134	42	26	34	28	67	30
Number of animals employed	0	1	29	20	25	7	15	21

Table IV.—The Percentages of Recoveries in Various Animals Infected with *T. brucei,* Zululand Strain, 1913.

	Horse.	Ox.	Goat.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Percentages	0	83	0	0	0	0	0	0
Number of animals employed	3	6	4	8	17	8	10	23

Compare this with the following table:—

Table V.—The Percentages of Recoveries in Various Animals Infected with the Trypanosome causing Disease in Man in Nyasaland.

	Horse.	Ox.	Goat and sheep.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Percentages	0	80	0	0	0	0	0	0
Number of animals employed	0	5	29	20	25	7	15	21

CONCLUSION.

The pathogenic action of *T. brucei*, Zululand strain, 1913, on various animals is so similar, not only in regard to the symptoms during life but also in the *post-mortem* appearances and rate of mortality, to that of the trypanosome causing disease in man in Nyasaland, that it affords another proof that these two trypanosomes are identical.

The Trypanosome causing Disease in Man in Nyasaland.
Part III.—*Development in* Glossina morsitans.

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Major A. E. HAMERTON, D.S.O., and Captain D. P. WATSON, R.A.M.C.; and Lady BRUCE, R.R.C. (Scientific Commission of the Royal Society, Nyasaland, 1912–14.)

(Received March 17,—Read March 26, 1914.)

[PLATE 24.]

INTRODUCTION.

In previous papers* the morphology of this trypanosome and the susceptibility of various animals to its pathogenic action have been described. In this is given an account of its development in *Glossina morsitans*.

In Uganda the study of the development of *Trypanosoma gambiense* in *G. palpalis* was much assisted by the circumstance that large numbers of laboratory-bred tsetse flies were available. This was due to the fact that the pupæ of *G. palpalis* could be collected on the lake-shore in practically unlimited numbers. It is quite otherwise with *G. morsitans*. It has been found impossible to find the pupæ of this species in any numbers, so that all laboratory-bred *G. morsitans* have had to be hatched out of pupæ obtained from captive flies, a slow and laborious process. The flies are caught some 20 to 30 miles from the laboratory and brought up to Kasu camp by a native on a bicycle. This kills a large number of the flies. Moreover, the climatic conditions at the camp are not always favourable for breeding and hatching out. This was remedied to some extent by establishing a breeding station down in the low-country, but as this had to be left in the charge of natives the results were not always very satisfactory.

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* 'Roy. Soc. Proc.,' B, vol. 85 (1912), and B, vols. 86 and 87 (1913).

The study of the development of this trypanosome in *G. morsitans* has therefore been rendered difficult by the small number of laboratory-bred tsetse flies which could be obtained. Over and above that, flies bred from captive flies are not so strong and healthy as those hatched out from wild pupæ.

An attempt was made to use wild flies by feeding batches of about 20 on healthy animals and picking out those cages which did not give rise to infection. But this is at best a roundabout and clumsy method, as it can never be certain, although every care is taken, that only clean flies are being dealt with.

The Development of the Trypanosome causing Disease in Man in Nyasaland in G. morsitans.

Eleven experiments were carried out with laboratory-bred flies. Three were positive and eight negative.

Five experiments were also carried out with wild flies, as no laboratory-bred flies were available. All were positive.

Tables I and II show these 16 experiments: the number of flies used; the number of infected flies found on dissection; and the number of days which elapsed before the flies became infective. As each fly died it was dissected and the result noted. As will be seen from Table I, several infected flies were found in the negative experiments. This probably means that the flies were only infected, not infective. The number of days before a fly becomes infective is arrived at by deducting seven days from the number of days which elapsed between the first infected feed of the flies and the

Table I.—Laboratory-bred Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Temperature at which flies kept.
1912.						
May 22	563	18	–	0		
June 13	668	22	–	1		
July 15	879	32	–	7		
„ 29	1003	28	+	2	31	84° F. (29° C.)
Aug. 17	1072	27	–	3		
Oct 23	1494	22	–	3		
Nov. 6	1560	19	–	0		
Dec. 13	1686	24	–	2		
„ 23	1710	30	–	0		
„ 30	1723	35	+	3	14	84° F. (29° C.)
1913.						
Aug. 31	2405	30	+	4	23	84° F. (29° C.)

appearance of trypanosomes in the blood of the experimental animal. Seven days is put down as the average number of days between the infection of the animal and the appearance of the trypanosomes in its blood—the incubation period. It is probably a day or two shorter.

The number of flies used in each experiment was small, due to the difficulty of obtaining laboratory-bred flies. They were kept during the experiment in the incubator at a temperature of 84° F. (29° C.).

In Experiment 1723 the number of days which elapsed before the flies became infective is only 14. This number is obtained, as mentioned above, by deducting seven days for the incubation period, but this may have been a day or two less. The flies were kept at an evenly warm temperature, which would tend materially to shorten the period of development. Still, 14 days seems a short time to elapse between the first feed on the infected animal and the appearance of an infective fly in the cage.

Two hundred and eighty-seven laboratory-bred flies were used and 25 infected flies were found—8·7 per cent.

Table II.—Wild Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Temperature at which flies kept.
1912.						
Dec. 11	1680	80	+	8	18	84° F. (29° C.)
„ 13	1688	40	+	6	3	84° F. (29° C.)
„ 18	1705	45	+	7	1	84° F. (29° C.)
1913.						
Jan. 9	1748	70	+	1	25	84° F. (29° C.)
„ 14	1729	20	+	1	30	84° F. (29° C.)

Experiments 1688 and 1705 are evidently cases of infection by naturally-infected wild flies which had escaped detection. They are included in the table as they both show invasion of the salivary glands and so help to throw light on the mode of development of this trypanosome in *G. morsitans.* The other three pass through an interval of 18, 25, and 30 days before the cages became infective. These are probably cases where there was no naturally-infected fly in the cage, and these periods therefore represent the usual length of time required for the cycle of development of this trypanosome to take place in *G. morsitans.* The wild flies were also kept in the incubator at a temperature of 84° F.

Two hundred and fifty-five flies were used and 23 infected flies were found —9 per cent.

Details of the Eight Positive Experiments.

The following table gives the details of the eight positive experiments :—

Table III.

Expt.	Day of expt.	Procedure.	Remarks.
1003	1–2 3 4–41	Flies fed on infected dog. Starved. Fed on clean Monkey 1023.	Trypanosomes appeared in blood of Monkey 1023 on the 38th day.
1723	1–4 5 6–22	Flies fed on infected dog. Starved. Fed on clean Monkey 1733.	Trypanosomes appeared in blood of Monkey 1733 on the 21st day.
2405	1–6 7 8–32	Flies fed on infected monkey. Starved. Fed on clean Monkey 2410.	Trypanosomes appeared in blood of Monkey 2410 on the 30th day.
1680	1–2 3 4–22	Flies fed on infected dog. Starved. Fed on clean Dog 1708.	Trypanosomes appeared in blood of Dog 1708 on the 25th day.
1688	1–2 3 4–12	Flies fed on infected monkey. Starved. Fed on clean Monkey 1699.	Trypanosomes appeared in blood of Monkey 1699 on the 10th day.
1705	1–2 3 4–9	Flies fed on infected monkey. Starved. Fed on clean Monkey 1707.	Trypanosomes appeared in blood of Monkey 1707 on the 8th day.
1748	1–2 3 4–30	Flies fed on infected monkey. Starved. Fed on clean Monkey 1845.	Trypanosomes appeared in blood of Monkey 1845 on the 32nd day.
1729	1–2 3 4–38	Flies fed on infected dog. Starved. Fed on clean Dog 1767.	Trypanosomes appeared in blood of Dog 1767 on the 37th day.

Omitting Experiments 1688 and 1705, it would appear from the remaining six experiments that an average period of 24 days is required to complete the cycle of development of the trypanosome causing disease in man in Nyasaland in *G. morsitans*, the flies being kept at a temperature of 84° F.

Details of the Eight Negative Experiments.

The following table shows the method of procedure in carrying out the eight negative experiments :—

Table IV.

Expt.	Day of expt.	Procedure.	Remarks.
563	1–3 4 5–52	Flies fed on infected monkey. Starved. Fed on clean Monkey 594.	All flies negative on dissection.
668	1–2 3 4–63	Flies fed on infected dog. Starved. Fed on clean Dog 699.	One infected fly found on the 42nd day.
879	1–2 3 4–32 33–63	Flies fed on infected monkey. Starved. Fed on clean Monkey 910. Fed on clean Monkey 1073.	Seven infected flies found.
1072	1–3 4 5–54	Flies fed on infected dog. Starved. Fed on clean Dog 1148.	Three infected flies found.
1494	1–3 4–5 6–44	Flies fed on infected monkey. Starved. Fed on clean Monkey 1514.	Three infected flies found.
1560	1–3 4 5–37	Flies fed on infected monkey. Starved. Fed on clean Monkey 1581.	All flies negative on dissection.
1686	1–4 5 6–43	Flies fed on infected monkey. Starved. Fed on clean Monkey 1704.	Two infected flies found.
1710	1 2 3–47	Flies fed on infected dog. Starved. Fed on clean Monkey 1718.	All flies negative on dissection.

RESULT OF THE DISSECTION OF THE INFECTED FLIES.

All the flies dying during the progress of these experiments were dissected.

In the three positive experiments with the laboratory-bred flies nine infected flies were found. The following table gives the results of the dissection of these nine flies. The second column gives the number of days which elapsed between the fly's first infected feed and its death and dissection. In the third column the labial cavity and hypopharynx are included under "Proboscis." At the time these experiments were made no attempt was made to distinguish between the two parts, as has been done lately in the case of *T. simiæ*.* When the proboscis is marked positive, as in Table VI, it may be that the trypanosomes are contained in the labial cavity or the hypopharynx, or both.

In the development of *T. gambiense* in *G. palpalis* trypanosomes were never noted as occurring in the proboscis.* In this species they are noted on several occasions as occurring in this position, but only in the wild-fly experiments, not in the laboratory bred. It seems natural to expect that if the salivary glands are swarming with trypanosomes that some of them will sometimes appear in the hypopharynx and, moreover, in the wild flies some of the infections of the proboscis are no doubt due to *T. pecorum*, *T. simiæ* or *T. capræ*, all of which develop in the proboscis.

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* 'Roy. Soc. Proc.,' B, vol. 87, p. 59 (1913).

Table V.—Laboratory-bred Flies. Result of the Dissection of the Infected Flies found in the Positive Experiments.

Expt.	Time, days.	Proboscis.	Proventriculus.	Fore-gut.	Mid-gut.	Hind-gut.	Salivary glands.
1003	33	–			+		–
1003	39	–			+		?
1723	30	–	+ +	+ +	+ +	+ +	–
1723	30	–	+ +	+ +	+ +	+ +	–
1723	48	–	–	–	–	–	–
2405	32				+		–
2405	33	–	+ +	+ +	+ +	+ +	+ +
2405	33	–	–	+	+	+	–
2405	33	–	–	+	+	+	–

In Experiment 1003, two infected flies were found. The first had only a gut infection and, unfortunately, it was found impossible to dissect out the salivary glands of the second. Neither had an infection of the proboscis.

In Experiment 1723, three infected flies were found. The first and second had the alimentary tract swarming with flagellates, but none in the salivary glands. The third was found on dissection to be free from trypanosomes throughout. This is curious because this fly had been isolated in a glass tube as an infective fly, and had, when used alone on a rat and rabbit, infected both these animals. The fly remained alive in the tube for 13 days, and the only explanation that can be given is that in this case the trypanosomes disappeared absolutely from the fly some few days before its death. This was the first time this had been observed to take place, and it was thought to be a remarkable phenomenon and difficult to credit, until another example of the same kind was observed. It must, therefore, be held as probable that an infective fly, with presumably both salivary glands and alimentary tract swarming with trypanosomes, can lose all these flagellates and become non-infective.

In Experiment 2405, four infected flies were found. Three of these were infections limited to the gut. The fourth was a good example of a salivary-gland infection. The glands were swarming with trypanosomes, and a portion of one of them injected under the skin of Rat 2417 gave rise to infection.

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* *Ibid.*, B, vol. 82 (1910).

Table VI.—Wild Flies. Result of the Dissection of the Infected Flies found in the Positive Experiments.

Expt.	Time, days.	Proboscis.	Proventriculus.	Fore-gut.	Mid-gut.	Hind-gut.	Salivary glands.
1680	5	−	−		+		−
1680	19				+		−
1680	32	+	+ +	+ +	+ +	+ +	+ +
1680	32	−	−	+	+	+	−
1680	33	−	+ +	+ +	+ +	+ +	−
1680	33	−	−		+		−
1680	33	−	−	+ +	+ +	+ +	−
1680	33	−	−	+	+	−	−
1688	10	−			+		−
1688	10	−	−	−	+		−
1688	11	−	−	−	+		−
1688	13	−			+		−
1688	15	−	+ +	+ +	+ +	+ +	+ +
1688	15	+	−		+		−
1705	8	+	+	+	+		−
1705	8	−	+	+	+		−
1705	10	−		−	+		−
1705	11	−		+			−
1705	12	+	+	+	+	+	+ +
1705	26	−	−	+ +	+ +	+ +	+ +
1705	33	−	+	+ +	+ +	+ +	+ +
1748	31	−	−	+ +	+ +	+ +	+ +
1729	48	+	+	+	+	+	+ +

In Experiment 1680, eight flies were found to be infected. In seven the flagellates were confined to the alimentary tract. The eighth had a well-marked invasion of the salivary glands. In this case trypanosomes were also seen in the proboscis, but whether in the labial cavity or the hypopharynx is not specified.

In Experiment 1688, six flies were found to contain trypanosomes in the alimentary canal. In one of these there was also infection of the salivary glands, which were crowded with trypanosomes. This fly must have been naturally infected when caught, as sufficient time had not elapsed since the infected feed to allow of time for development to take place. The flagellates contained in the salivary glands injected into Rat 1721 gave rise to infection.

In Experiment 1705, seven infected flies were found. Three of these had the salivary glands invaded. One of these, the fifth, must also have been a naturally-infected wild fly.

In Experiment 1748, only one infected fly was found. It had a copious infection of the salivary glands, a portion of which injected into Rat 1852 gave a positive result.

In the last Experiment, 1729, there was also only one infected fly found. The salivary glands were swarming with trypanosomes.

The next table gives the result of the dissection of the infected flies found in the experiments which remained negative.

In the negative Experiments 563, 1560, and 1710, none of the flies were found to be infected with trypanosomes in any part (see Table I). These experiments are therefore omitted from this table.

Table VII.—Laboratory-bred Flies. Result of the Dissection of the Infected Flies found in the Negative Experiments.

Expt.	Time, days.	Proboscis.	Proventriculus.	Fore-gut.	Mid-gut.	Hind-gut.	Proctodæum.	Salivary glands.
668	42	–		+	+	+		–
879	7	–	–	+	+	+		–
879	8	–		+	+ +			–
879	9	–		+	+	–		–
879	11	–			+			
879	24	–	+	+	+	+	–	–
879	28	–	+ +	+ +	+ +	+ +		–
879	40	–	+ +	+ +	+ +	+		–
1072	7	–	–	+	+	+		–
1072	10	–		+	+	+		
1072	38	–	+ +	+ +	+ +	+ +		+ +
1494	7	–	+ +	–	–	–		–
1494	17	–		+	+	–		–
1494	31	–		+	+			–
1686	8	–	–	+ +	+ +	+		–
1686	26		–	+	+	+		–

From these negative experiments it will be seen that only in one fly did an infection of salivary glands occur. Why this fly did not infect the animal it fed on is impossible to say.

THE METHODS USED IN THE EXAMINATION OF THE FLIES.

The flies were dissected as described in a previous paper.* As each fly in a cage died it was dissected, and the result, as regards the presence of trypanosomes in the alimentary tract and salivary glands, recorded. Fixed and stained preparations were then made from the various parts and numerous drawings of the various types of trypanosomes encountered were made. The method described in a previous paper† of isolating infective flies and inducing them to salivate on clean cover-glasses was also made use of. This is a useful, simple and practical method, as it demonstrates clearly the type of trypanosome thrown out from the tip of the proboscis when the fly feeds.

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* 'Roy. Soc. Proc.,' B, vol. 83, p. 513 (1911).

† *Ibid.*, B, vol. 87, p. 63 (1913).

THE TRYPANOSOMES FOUND IN THE ALIMENTARY TRACT.

In this species of trypanosome the developmental changes which take place in the intestine of *G. morsitans* are similar to those already described as occurring in the development of *T. gambiense* in *G. palpalis*.* The latter development has also been worked out very fully and completely by others.† It is therefore unnecessary here to do more than refer to these previous descriptions as being equally applicable to the species under consideration.

In this species of trypanosome also, as in *T. gambiense*, it is only a small percentage of the flies fed on an infected animal which become infected. In one series of *T. gambiense* this was 8 per cent.‡ In this species the experiments with laboratory-bred flies was 8·7 per cent., with wild flies 9 per cent. Just as in *T. gambiense*, the development takes place in the alimentary tract and salivary glands and not in the proboscis.

THE TRYPANOSOMES FOUND IN THE SALIVARY GLANDS.

In the trypanosome causing disease in man in Nyasaland, as in *T. gambiense*, the crux of the whole matter is the invasion of the salivary glands. After a certain number of days—in this species from 14 to 31—the trypanosomes reach the salivary glands and the fly becomes infective.

Plate 24, figs. 3–28, represent the various stages in the development of this trypanosome in the salivary glands. Figs. 1 and 2 are trypanosomes from the proventriculus; these represent the dominant intestinal type, from which the salivary-gland types arise. It is still a matter of speculation as to how they gain access to the glands, but as described in a former paper,§ there is no doubt they are often thrown forward into the proboscis during or just in the act of feeding, and may, under these conditions, be drawn into the hypopharynx and so reach their destination. These proventricular forms, however, have never been actually seen by the Commission in the hypopharynx. Figs. 3–11 are forms found in the salivary glands. Many of these are crithidial in type and occur in numbers. Figs. 12–14 are what appear to be encysted forms. Figs. 16–21 are "blood forms" and occurred in large numbers in the same preparation as the crithidial type shown in figs. 3–8. Figs. 22–28 are "blood forms" which were thrown out on to a cover-glass by a living infective fly. The preparation was beautifully clear, each individual trypanosome standing out distinctly. Fig. 23 is from the same preparation

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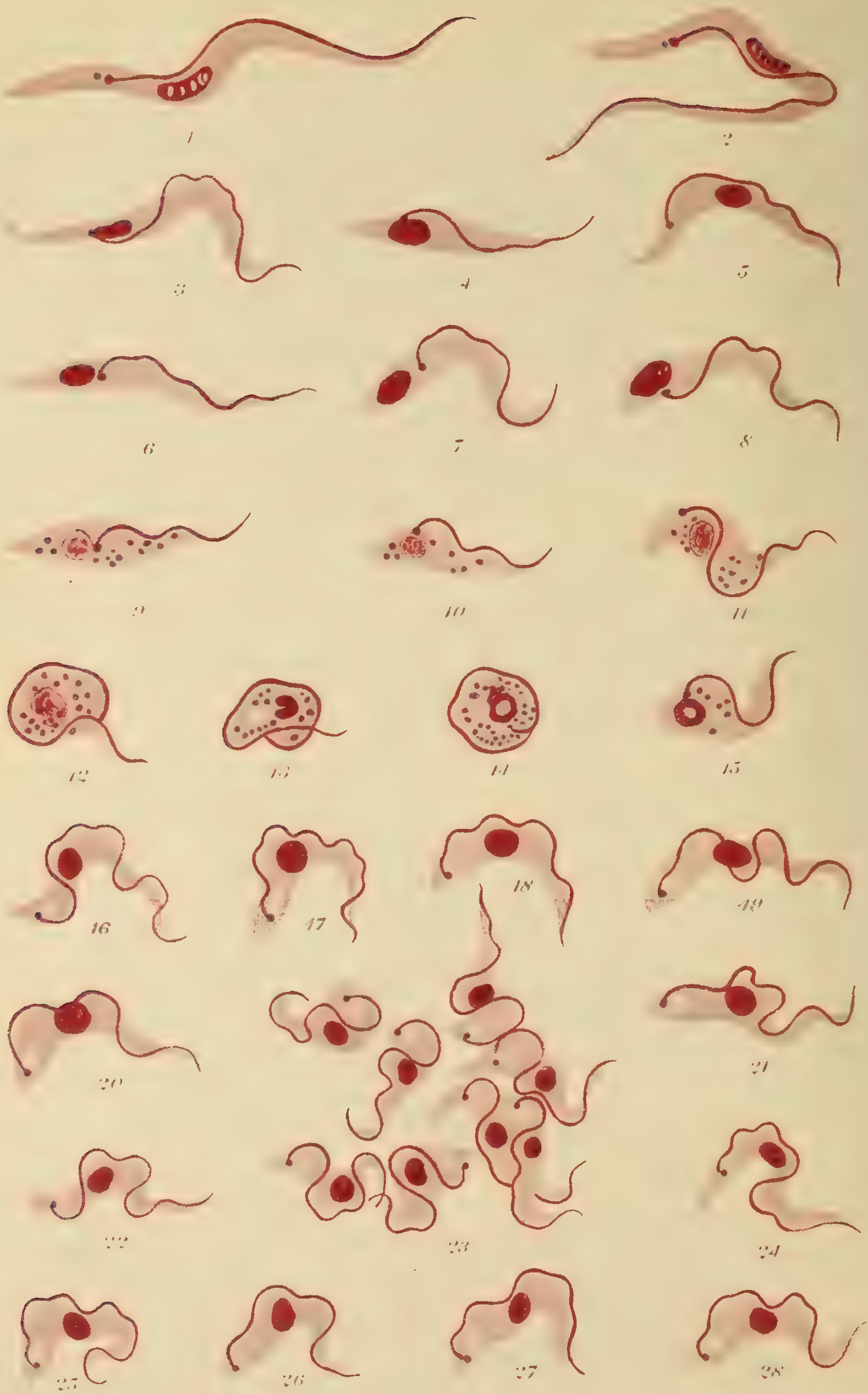
* *Ibid.*, B, vol. 83, p. 515 (1911).

† Muriel Robertson, M.A., 'Phil. Trans.,' B, vol. 203 (1913).

‡ 'Roy. Soc. Proc.,' B, vol. 83, p. 514 (1911).

§ *Ibid.*, B, vol. 87, p. 65 (1913).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Trypanosome Causing Disease in Man in Nyasaland.
Development in Glossina morsitans.

X 2000

M. E. Bruce, del.

and has the appearance of a small bunch or clump of "blood forms" in the act of breaking apart.

CONCLUSIONS.

1. The trypanosome causing disease in man in Nyasaland belongs to the same group as *T. gambiense,* the development taking place in the alimentary tract and salivary glands, not in the proboscis, of the fly.

2. The percentage of flies which become *infected* is the same as in *T. gambiense,* 8 per cent.

3. The percentage of flies which become *infective* is about 1 per cent.

4. The length of time which elapses before a fly becomes infective varies from 14 to 31 days, average 23 days.

5. The infective type of trypanosome in the salivary glands—corresponding to the final stage of the cycle of development—is similar to the short and stumpy form found in the blood of the vertebrate host.

DESCRIPTION OF PLATE.

Figs. 1–2.—Trypanosomes from proventriculus. These represent the dominant intestinal type.

Figs. 3–8.—Trypanosomes taken from a preparation of the salivary gland of an infective fly. Many of these are crithidial in type, *e.g.,* figs. 6, 7, and 8.

Figs. 9–15.—Other forms seen in the salivary glands. Figs. 12–14 have the appearance of being encysted.

Figs. 16–21.—The fully developed "blood forms." Without these the fly is non-infective. These were drawn from the same preparation as figs. 3–8.

Figs. 22–28.—Trypanosomes ejected by a living infective *G. morsitans* on attempting to feed through a cover-glass. Fully developed "blood forms."

Stained Giemsa. × 2000.

Description of a Strain of Trypanosoma brucei *from Zululand.*
Part III.—*Development in* Glossina morsitans.

By Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S.; Major A. E. Hamerton, D.S.O., and Captain D. P. Watson, R.A.M.C.; and Lady Bruce, R.R.C. (Scientific Commission of the Royal Society, Nyasaland, 1912–14.)

(Received March 17,—Read March 26, 1914.)

[Plate 25.]

Introduction.

In previous papers* the morphology of this trypanosome and its action on animals were described. The chief object of this paper is to compare the development of this species of trypanosome with that of the trypanosome causing disease in man in Nyasaland—the *T. rhodesiense* of Stephens and Fantham. The development of the latter has already been described.† It will therefore only be necessary here to present the various data in the form of tables and figures, which can then be compared with similar tables and figures in the previous paper.

The Development of T. brucei, Zululand Strain, 1913, in G. morsitans.

Seven experiments were made with laboratory-bred flies. Three of these were positive and four negative. Two experiments were also made with wild flies, both of which were positive.

Table I.—Laboratory-bred Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Temperature at which flies kept.
1913.						
Feb. 11	1857	58	–	1		
,, 17	1909	50	+	10	21	?
Mar. 11	1988	45	+	20	13	84° F. (29° C.)
,, 17	1996	55	–	4		
April 25	2111	50	–	3		
May 26	2188	30	–	1		
June 23	2188A	20	+	8	14	84° F. (29° C.)

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* 'Roy. Soc. Proc.,' this vol., pp. 493 and 511.

† *Ibid.*, this vol., p. 516.

Three hundred and eight flies were used and 47 infected flies were found—15·3 per cent.

It is difficult or impossible to explain the difference in the ratio of infected flies. Experiment 1857 has only one infected fly in 58; Experiment 1988, 20 in 45. There is no record as to whether Cage 1857 was kept in the incubator or not, but it is to be presumed that it was, as was the habit at that date. From Table III it will be seen that the flies in Experiment 1988 were fed for eight days on an infected dog, monkey, and goat. It is possible that this had something to do with the high rate of infection, but it is impossible to say with certainty. The scarcity of laboratory-bred flies made it out of the question to pursue this line of inquiry. Experiment 2188 has also only one infected fly in 30, but this is capable of explanation. Experiments 2188 and 2188A were carried out for the sake of economy with the same cage of flies. It having become evident (see Table IV) that the flies after their first feeding on an infected rat had failed to infect Monkey 2203, the 20 remaining flies were again fed on an infected guinea-pig, with the result that eight of them became infected.

Table II.—Wild Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Temperature at which flies kept.
1913. July 22	2309	50	+	0	34	84° F. (29° C.)
„ 26	2313	50	+	5	24	84° F. (29° C.)

In Experiment 2309 none of the flies were dissected, hence no infected flies were found. In Experiment 2313, only 21 flies out of 50 were dissected. These experiments are tabulated here as they give the number of days before the flies became infective, and thus afford data as to the length of time the cycle of development runs in the fly.

Details of the Five Positive Experiments.

The following table gives the principal details in the carrying out of the five positive experiments. The first three were carried out with laboratory-bred flies, the last two with wild flies.

It would appear from these five experiments that an average period of 21 days elapses before the cycle of development of *T. brucei*, Zululand, 1913, is complete in *G. morsitans* and the fly becomes infective.

Table III.

Expt.	Day of expt.	Procedure.	Remarks.
1909	1–9	Flies fed on infected monkey.	Trypanosomes appeared in blood of Monkey 1970 on the 28th day.
	10	Starved.	
	11–29	Fed on clean Monkey 1970.	
1988	1–8	Flies fed on infected dog, monkey, and goat.	Trypanosomes appeared in blood of Dog 2007 on the 20th day. Monkey 2058 showed trypanosomes on the 30th day.
	9	Starved.	
	10–22	Fed on clean Dog 2007.	
	23	Starved.	
	24–29	Fed on clean Monkey 2058.	
2188A	1–9	Flies fed on infected guinea-pig.	Trypanosomes appeared in blood of Dog 2254 on the 21st day. Monkey 2298 showed trypanosomes on the 32nd day.
	10	Starved.	
	11–22	Fed on clean Dog 2254.	
	23	Starved.	
	24–27	Fed on clean Monkey 2298.	
2309	1–7	Flies fed on infected dog.	Trypanosomes appeared in blood of Monkey 2316 on the 41st day.
	8	Starved.	
	9–42	Fed on clean Monkey 2316.	
2313	1–4	Flies fed on infected monkey.	Trypanoeomes appeared in blood of Dog 2361 on the 31st day. Monkey 2318 never showed trypanosomes.
	5	Starved.	
	6–15	Fed on clean Monkey 2318.	
	16	Starved.	
	17–31	Fed on clean Dog 2361.	

Details of the Four Negative Experiments.

The following table shows the method of procedure in carrying out the four negative experiments. In each of them laboratory-bred flies were used:—

Table IV.

Expt.	Day of expt.	Procedure.	Remarks.
1857	1–8	Flies fed on infected monkey.	One infected fly found on the 62nd day.
	9	Starved.	
	10–60	Fed on clean Monkey 1941.	
1996	1–9	Flies fed on infected monkey.	Four infected flies found.
	10	Starved.	
	11–55	Fed on clean Monkey 2031.	
2111	1–8	Flies fed on infected dog.	Three infected flies found.
	9	Starved.	
	10–14	Fed on clean Dog 2100.	
	15	Starved.	
	16–30	Fed on clean Monkey 2125.	
	31	Starved.	
	32–43	Fed on clean Dog 2189.	
2188	1–8	Flies fed on infected rat.	One infected fly found on the 21st day.
	9	Starved.	
	10–27	Fed on clean Monkey 2203.	

RESULT OF THE DISSECTION OF THE INFECTED FLIES.

Table V.—Laboratory-bred Flies. Positive Experiments.

Expt.	Time, days.	Proboscis.		Proventriculus.	Crop.	Fore-gut.	Mid-gut.	Hind-gut.	Salivary glands.
		Labial cavity.	Hypopharynx.						
1909	33	−				+			−
1909	34	−		+		+ +	+ +	+ +	−
1909	35	−		−		+ +	+ +	+ +	+ +
1909	36	−		+ +		+ +	+ +	+ +	+ +
1909	37	−				+	+	+	−
1909	43	−				+ +	+ +		−
1909	47	−		+			+		−
1909	50	−					+		−
1909	51	−	−			+ +	+ +		+ +
1909	57			−			+		−
1988	22	−	−		+ +	+ +	+ +	+ +	−
1988	23	−	−	−	−	−	+	+	−
1988	30	−	−	+		+	+		−
1988	30	−	−	−			+		−
1988	31	−	−				+		−
1988	31	−	+	+	+	+	+	+	+ +
1988	31	+	−	+		+	+	+	−
1988	33	−	−				+		−
1988	34	−	−				+		−
1988	34			+		+	+	+	+
1988	34	−	−				+		−
1988	34	−	−				+		−
1988	34	−	−				+		−
1988	34	−	−				+		−
1988	34	−	−				+		−
1988	34	−	−	+			+		−
1988	35	−	−				+		−
1988	36	−	−	+		+	+	+	−
1988	37	−	−			+	+	+	−
1988	37	−	−	+		+	+	+	−
2188A	24	−	−				+		−
2188A	24	−	−				+		−
2188A	29						+		−
2188A	32						+		−
2188A	32						+		−
2188A	32						+		−
2188A	32					+	+	+	+ +
2188A	32						+		−

In Experiment 1909, 10 infected flies were found. Three of these had the salivary glands swarming with trypanosomes; in none was the labial cavity or hypopharynx found to contain flagellates.

In Experiment 1988, 20 infected flies were found. Two had an invasion of the salivary glands. In one it is noted that a few active trypanosomes were seen in the labial cavity, and in one that a few "blood forms" were seen in the hypopharynx.

In Experiment 2188A, eight infected flies were found, one of which had the salivary glands swarming with trypanosomes. Parts of these glands injected into Rats 2311 and 2312 gave in both cases a positive result.

Table VI.—Laboratory-bred Flies. Negative Experiments.

Expt.	Time, days.	Proboscis.		Proventriculus.	Crop.	Fore-gut.	Mid-gut.	Hind-gut.	Salivary glands.
		Labial cavity.	Hypopharynx.						
1857	62	–	–	–			+		–
1996	23					+ +	+ +	+ +	–
1996	43			–	–	+	+	+	–
1996	57	–	–	–		+ +	+ +	+ +	–
1996	57						+ +		–
2111	12	–	–	–	–	–	+	+	–
2111	29	–	–	+	+	+ +	+ +	+ +	–
2111	40	–	–				+		–
2188	21					–	+	+	–

In none of the negative experiments was an infection of the salivary glands found. Nine infected flies were dissected, but not one of these had passed into the infective stage. No parasites were found in the proboscis at any time.

From a consideration of these tables it will be seen that this strain of *T. brucei*, Zululand, 1913, belongs to the same group as *T. gambiense* and the trypanosome causing disease in man in Nyasaland as far as their development in *G. palpalis* and *G. morsitans* is concerned. This development takes place in the intestine of the fly and, after a varying number of days, passes forward into the salivary glands, where the final stage in the cycle is reached—the infective or "blood forms." In this group the parasites are never found fixed in the labial cavity as in the *pecorum* and *vivax* groups.

THE TYPE OF TRYPANOSOME FOUND IN THE INFECTED FLIES.

Plate 25 represents the developmental forms of *T. brucei*, Zululand, 1913, found in the proventriculus and salivary glands of *G. morsitans*. A description of the various types found in the different parts of the alimentary tract is not considered necessary, as they are identical with those found in the development of *T. gambiense* in *G. palpalis*, which have already been fully described.*

Figs. 1 and 2 are two long trypanosomes from the proventriculus. Figs. 3–6 are the same, but they were extruded on to a cover-glass by

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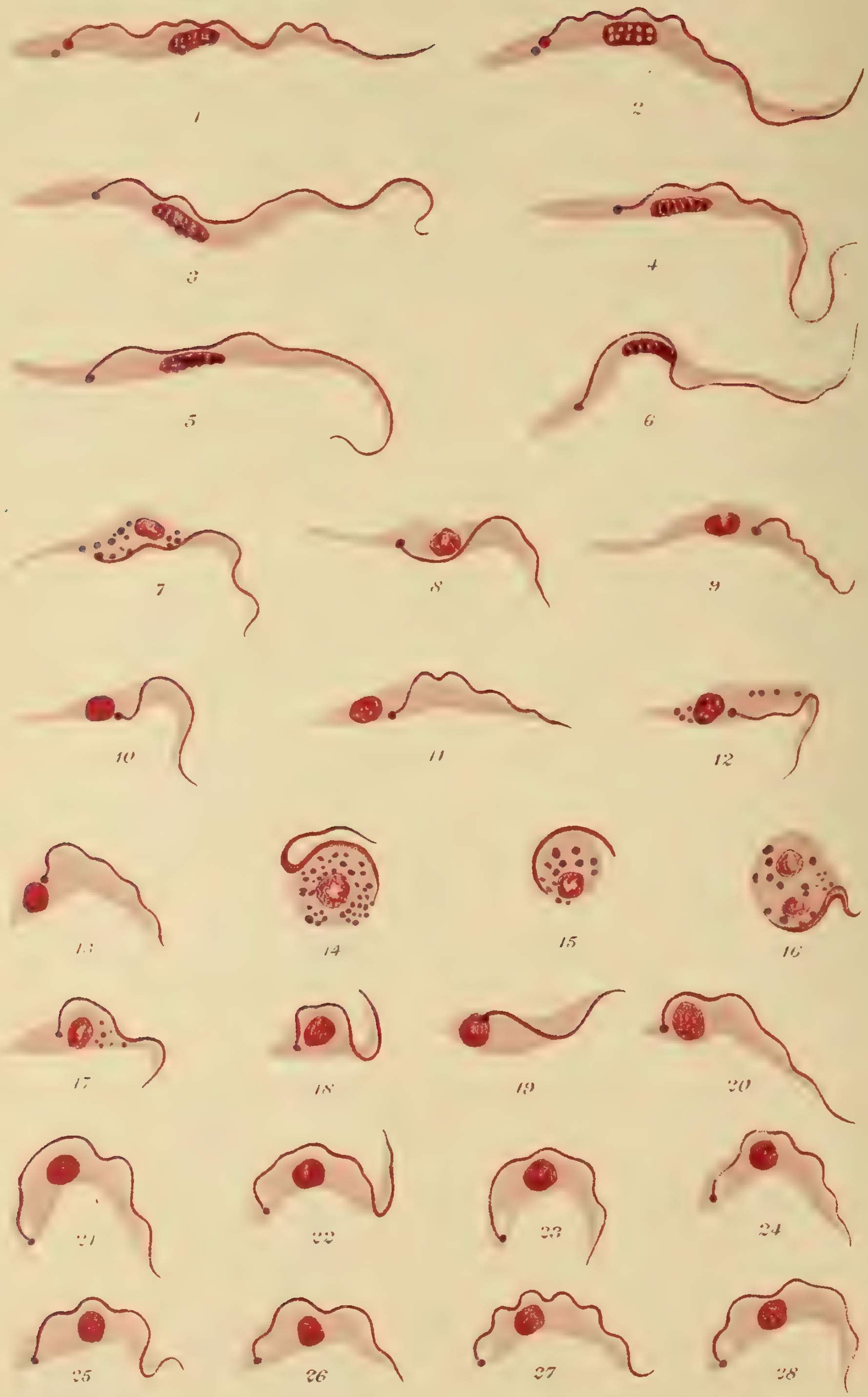
* 'Roy. Soc. Proc.,' B, vol. 83 (1911).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Trypanosoma brucei. Zululand 1913.
Development in Glossina morsitans.

M. E. Bruce. del.

X 2000.

a living non-infective fly, and probably came through the labial cavity from the proventriculus. Some of them may possibly have come from the hypopharynx, and in that case they may have been proventricular forms on their way to infect the salivary glands. Figs. 7–20 are various types found in the salivary glands—crithidial, encysted, and immature "blood forms." Figs. 21–28 represent the finished product—the infective or "blood forms."

If this plate is compared with that representing the developmental forms of the trypanosome causing disease in man in Nyasaland* in the proventriculus and salivary glands of *G. morsitans*, the extraordinary likeness between the two is at once evident, and is another argument in favour of these two strains being identical.

CONCLUSIONS.

1. *T. brucei*, Zululand, 1913, belongs to the same group as *T. gambiense* as regards its cycle of development in the tsetse fly.

2. It has been shown that the trypanosome causing disease in man in Nyasaland also belongs to the same group.

3. The cycle of development of the Nyasaland and Zululand trypanosomes in *G. morsitans* is so marvellously alike that it affords another reason for believing in the identity of these two trypanosomes.

DESCRIPTION OF PLATE.

Figs. 1 and 2.—Trypanosomes from proventriculus.

Figs. 3–6.—Proventricular types which were extruded on to a cover-glass by a living non-infective fly.

Figs. 7–20.—Crithidial, apparently encysted, and immature "blood forms" found in the salivary glands.

Figs. 21–28.—The final stage in the salivary glands—the infective or "blood forms."

Stained Giemsa. × 2000.

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* 'Roy. Soc. Proc.,' this vol., Plate 24.

On the Lack of Adaptation in the Tristichaceæ and Podostemaceæ.

By J. C. Willis, M.A., Sc.D., etc., Director of the Botanic Gardens,
Rio de Janeiro.

(Communicated by Dr. D. H. Scott, F.R.S. Received February 24,—
Read April 30, 1914.)

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With the exception of some of the parasitic orders, such as the *Balanophoraceæ*, there are probably no families of flowering plants—one might almost include flowerless—which are so completely transformed from the average or mesophytic type of the phanerogams into types which are so completely unique and peculiar, as the Tristichaceæ and still more the Podostemaceæ. Nor are there any in which, with such very great uniformity in the conditions of life, there is such remarkable variety in the morphological structure.

The structure of the orders, or rather of their members, being unique, and the conditions under which they live being also unique, it has been taken for granted that the former is in a high degree adapted to the latter, the flat thallus-like expansions of stem or root being looked upon as admirably suited to the rushing water in which they live. So long as we were almost completely ignorant of the actual living plants, and content with dead material collected mainly in the dry seasons, this was all very well, but now that for 17 years I have devoted much attention to these plants,* have studied them in the living condition in their natural habitats in India, Ceylon, and Brazil, have followed them from germination right through their life-history, and in other ways become absolutely familiar with them, and as a result of all this have arrived at diametrically opposite conclusions, it will repay us to examine into the question in some detail.

Evolution is usually supposed to have produced the extraordinary variety of forms now existing by adapting members of very different families to very different conditions of life. The climate, the soil, the competition with other living beings, many conditions, vary in the most complicated way, and it is very difficult, if not impossible, to disentangle the effect of the different factors that may have a hand in producing the result. But in the case of these two closely allied families we are able to study the problem of evolution with many of the complication of factors removed or simplified. There is no competition with other families, for these two have their habitats to themselves. There is no difference in soils, for all grow on naked waterworn rock. There is no difference in climate, for all grow in water in the tropical or sub-tropical zone, and are represented only by seeds in the colder weather. There is no difference in circumambient medium, for all grow in running water. The illumination is the same for all. The conditions of their life are the most absolutely uniform that it is possible to conceive; even in a laboratory it would be very difficult to produce conditions more uniform, and they must have been the same since the original founders of the orders first took to life in running water.

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* Willis, "A Revision of the Podostemaceæ of India and Ceylon," 'Ann. Perad.,' vol. 1, p. 181 (1902); "Studies in the Morphology and Ecology of the Podostemaceæ of Ceylon and India," *loc. cit.*, p. 267.

We cannot for one moment suppose that these plants took to the water at different stages of evolution into a family upon land; they must obviously have gone through the whole of their evolution in water from an extremely early stage, during which the ancestors probably retained a certain power of surviving upon land. Now the most essential point of this argument is that here we have two entire families, containing about 30 genera and over 100 species of the most varied morphological structure possible, entirely evolved under perfectly uniform conditions which cannot have varied, except for all alike,* since the evolution of the families began. The evolution consequently cannot have been in any sort of response to a necessity of adaptation to different conditions, for there are and have been no different conditions to which to be adapted, since the first members of these families began to live in running water.

We shall see, first of all, that the conditions of life are absolutely uniform (2) that the families contain the most astonishing variety of morphological structure, (3) that all the different stages, so to speak, in the attainment of the most bizarre of these forms, live together, and that the least bizarre are the most widespread and common, and in consequence (4) that as there are no changed conditions to which to be adapted, there can be no adaptation to conditions other than to the general conditions which are common to all, and have been common to them since the families began, and (5) that therefore there can, in all probability, be no selection of infinitesimal variations, but progress must have been by something of the nature of mutations or fixed changes without natural selection. Lastly we shall consider in brief the factors which seem to have had an influence in the evolution of these remarkable families.

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* As, for instance, if the climate became warmer.

The orders Tristichaceæ and Podostemaceæ (for I have shown in a recent paper* that the old family of Podostemaceæ must be split into these two, unless some intermediates can be found in Africa), as is well known, consist only of annual plants which live upon the surface of smooth water-worn rocks in rapidly moving water, and are confined, with the exception of one species found in Ohio, to the warmer regions of the globe. During the wetter season of the year they carry out their vegetative life, and form their flowers as the dry weather approaches. The flowers open above the water level as the rivers sink, and the seeds are shed upon the rocks, where those few that manage to retain their position germinate with the beginning of the wetter season.

Conditions of Life.—It has long been an axiom that the conditions of life of the plants of still water are extraordinarily uniform, and that to this is due the small number of these plants, and the small amount of evolution through which they appear to have gone and to be going, together with their enormously wide distribution. But it will soon be seen that the conditions of life for the Podostemaceæ and Tristichaceæ are if anything even more uniform, and yet in contrast to the plants of still water they show the most astounding morphological differences, and form about 30 genera with over 100 very different species (and it is certain that very many species and probably genera have yet to be discovered). The distribution of these species is more and more localised the more specialised they are in structure, while the widely distributed genera are those which are the most like the original ancestors from which the Podostemaceæ and Tristichaceæ are descended.

The conditions of life of these plants are, and must always have been, as uniform as, or more uniform than, those of other water plants. In the first place, as to substratum, they grow as a rule only upon rocks, but, as they will also grow upon anything which may have become wedged between the rocks so as to be immovable, the actual chemical or physical composition of the substratum cannot be of importance; in all probability the plants take nothing from it unless, perhaps, silica. They grow upon this rock bottom by means of creeping roots, which give off secondary shoots. They are thus absolutely compelled from the start to a plagiotropic mode of life. In the second place, as to temperature conditions: the orders are, with the exception of *Podostemon ceratophyllum*, found in Ohio, confined to the tropics and sub-tropics, and the coldest water in which I have ever seen any was 57° F. (14° C.), in the Khasia Hills of Assam, the warmest 81° F. (27° C.). Their temperature conditions are thus more uniform than those of other water plants. Their conditions of light supply are also uniform. They live only from the water-level down to perhaps 50–75 cm. The medium in which they live, the running water of tropical rivers, is very uniform, and their food supply also, as it comes from the passing water. The only differences between them in the numerous localities in which they occur are therefore in the speed of the water current. The fastest water in which I have found any was in the Rio Piabanha, north of Petropolis, near to Rio de Janeiro, where *Lophogyne arculifera* was growing in water going from 2½ to 4 miles an hour, and the slowest was at Hakinda, near Peradeniya, in Ceylon, where *Farmeria metzgerioides* and *Podostemon subulatus* were growing in an eddy that moved perhaps half a mile in the hour. But, if this involved any difference in structure, there would surely be more "Zugfestigkeit" in the thalli of the more rapid water, and an examination of the various sections given in my monograph of the Indian forms will show that this is not so—some have it, some not.

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* Willis, "A New Natural Order of Flowering Plants—Tristichaceæ," 'Linn. Soc. Journ., Bot.' (in the press).

It is true that at any one actual spot, of, say, a square yard, on a rock, there are most commonly only one or two species to be found growing, but this does not mean that no others could grow there, but simply that each one has some slight preference in positions. Always there is more or less of intermixture. For instance, from my Indian monograph I may quote the various species, with those which were recorded as found together with them:—

Tristicha ramosissima: Griffithella Hookeriana.

Lawia zeylanica: Hydrobryum olivaceum, lichenoides, Farmeria metzgerioides, Podostemon subulatus, Dicræa stylosa, Griffithella Hookeriana.

Podostemon subulatus: Dicræa elongata, stylosa, Farmeria metzgerioides, Lawia zeylanica, Hydrobryum olivaceum.

Podostemon Barberi: Griffithella Hookeriana.

Dicræa elongata: Podostemon subulatus, Dicræa stylosa, Hydrobryum olivaceum.

Dicræa dichotoma: Hydrobryum olivaceum.

Dicræa Wallichii: Hydrobryum lichenoides.

Dicræa stylosa: D. elongata, Podostemon subulatus, Hydrobryum olivaceum, lichenoides, Lawia zeylanica, Griffithella Hookeriana.

Griffithella Hookeriana: Lawia zeylanica, Podostemon Barberi, Dicræa stylosa, Tristicha ramosissima.

Willisia selaginoides: Hydrobryum lichenoides.

Hydrobryum lichenoides: H. olivaceum, Willisia selaginoides, Lawia zeylanica, Dicræa Wallichii.

H. sessile: ?

H. olivaceum: Lawia zeylanica, Farmeria metzgerioides, Dicræa stylosa elongata, dichotoma, Hydrobryum lichenoides.

H. Griffithii: ?

Farmeria metzgerioides: Lawia zeylanica, Hydrobryum olivaceum, Podostemon subulatus, etc.

Farmeria indica: ?

When we remember that often a certain species does not grow in the same district as another species, and that these are only a few observations, it is pretty evident that almost any species could live with almost any other, or in almost any place. In Brazil I have found the most incongruous-looking species, such as *Tristicha hypnoides* and *Apinagia Riedelii*, or *Mniopsis Weddelliana* and *Mourera aspera*, growing side by side, intermixed, in two or three localities. Further, in the State of Rio, their habitat is nearly always shared to some extent with a moss, which appears to be able to survive on the dry rock if exposed to the air by the fall of the water.

As regards other conditions of life, it may be pointed out that these plants escape to a large extent from competition with other plants, for, except for an occasional moss or fern, nothing else is ever found on the rocks with them, and, owing to the enormous destruction of seed before germination, there is little competition among themselves. They are to some considerable extent attacked by such animals as can get at them, for they are very rich in starch towards the end of the season.

Their conditions of life, then, are in the very highest degree uniform, but in no sense can this be said of their morphological construction, which is the most various and complex that one can conceive. By no stretch of imagination can the variety in the conditions of life be made to fit one quarter of the variety of structure. While the conditions of life and the variations in those conditions are the same in Brazil, India, and Africa, and certain almost identical species occur in all these places, the general trend of the morphological construction in India is towards flattened primary roots, in Brazil towards flattened secondary shoots, and in Africa, so far as our at present extremely limited knowledge goes, sometimes, at any rate, towards flattened roots combined with tall stems.

Morphological Structure.—Before going further, as these plants are not very familiar to most botanists, it will be well to sum up in brief some of their morphological peculiarities. Taking first the family Tristichaceæ (Tristicha, Lawia, and Weddellina), be it noted first of all that the members of this family, though only, many of them, slightly modified from "common" types, occupy the same positions as the highly modified members of the Podostemaceæ proper, whether in swiftly moving or in slowly moving water. Now if such simple forms as these suffice to occupy the positions, there can be no adaptational need for the complex forms of the Podostemaceæ proper. But to return to the structure: the most "primitive"-looking species is undoubtedly *Tristicha ramosissima* Willis, which grows in South-West India. The primary axis of this plant is not known, but as the creeping roots are just like those of other forms, it may be taken for granted that it gives rise to creeping closely attached roots, running over the rocks in all directions. Upon these are borne endogenous "secondary" shoots, which in this plant grow out in a fairly normal way to a length of a foot or so. They are not dorsiventral, and bear the flowers, which also are not dorsiventral, but have the formula P3, A3, G(3), and are perfectly regular. A feature of the plant is the numerous "ramuli" or shoots of limited growth, which here are in no sense dorsiventrally arranged or constructed. Now in this plant the only adaptations, if adaptations they can be called, to the mode of life, are (1) the great and regular development of secondary shoots on creeping roots, and (2) of haptera or organs for clinging to the rock, and (3) the absence of intercellular spaces. But I have no hesitation in affirming that this plant could live quite comfortably in any of the localities occupied by the members of the two families, with the exception of those in which during the growing season the water was liable to be less than an inch or an inch and a half in depth. Life in shallower water is quite easy for another species of Tristicha—*hypnoides*. But in this one begins to see increasing dorsiventrality in the plant. Once placed upon a rock, in which downward growth is impossible, and dorsiventral or plagiotropic direction of growth ensured by the creeping roots, the impetus to dorsiventrality appears to be given, and the whole further evolution of both families seems to be chiefly in the direction of increasing dorsiventrality, both in the vegetative and the floral organs. In *T. hypnoides* the main secondary shoot begins to show some dorsiventrality and the ramuli have become very distinctly tristichous, with one upper and two more lateral rows of leaves. The flower is also dorsiventral in having lost the upper and one lower stamen. In a new species of Tristicha which has lately been discovered near to Rio by Snr. F. Tamandaré de Toledo, the ramuli are not only tristichous, but lie down flat upon the rock, and have the upper row of leaves smaller than the laterals, while the secondary shoots have become flat expanded "stem-thalli." Leaves are borne on the upper surface of the flat expansion (which lies flat upon, and is attached to, the rock), and ramuli as well as flowers on the margins. The flowers have only the two lower stamens. Now this species can live quite happily in 5–8 mm. of water, and again I may repeat that these three Tristichas could perfectly well occupy all the localities of the Tristichaceæ and Podostemaceæ, the conditions being identical throughout. In actual fact they, with the other species of the genus—*T. alternifolia*—do occur in a very great proportion of the places in which plants of these families have been found, throughout the distribution zone of the genus Tristicha, which is almost coterminous with that of the orders.

Weddellina, a genus confined to the north of South America, has a structure very similar to that of *T. ramosissima,* and a symmetrical flower.

Lawia, the remaining genus of this family, departs, so to speak, upon a side line which was indicated by the secondary shoots of *Tristicha sp. nov.,* but here it is the primary axis which flattens out into a broad prostrate thallus, which does not bear ramuli like the rest of the order and has symmetrical flowers like *T. ramosissima.* The genus is confined to the region from Ceylon to Bombay, and has only one species, *L. zeylanica,* which occurs in many forms, differing slightly, so far as can be observed, in every locality.

Before going on to deal with the Podostemaceæ proper, it may be well to repeat that the Tristichaceæ can, and do, live quite happily in every locality occupied by the other family, and that the genus Tristicha, the typical genus of the family, and the least modified (in one at least of its species), is the most widely spread of all the genera of both families. The whole family Podostemaceæ, with its remarkable morphological constructions, is therefore adaptationally unnecessary. All its extraordinary features are *de luxe,* and cannot have arisen in response to any need for adaptation to different conditions, for there are no different conditions to which to be adapted, and none of the highly modified forms is so successful, or so common, as *T. hypnoides,* which has only a very slight modification from the original type from which the family is descended. We cannot suppose that all these changes had to go on just to fit the family to live in running water, or the first members of it would never have been able to survive, and the Podostemons, etc., which are only slightly modified, would not be the most widespread and common of all to-day.

To pass on now to the Podostemaceæ proper (tribe Achlamydatæ of the older writers), we may run very briefly over the striking morphological differences to be found in the family. They are mainly of two kinds—flattening of the secondary shoots and flattening of the primary roots. The least modified types in many respects are the South American forms Ligea (Œnone), Marathrum, and Mourera. All show, however, rather more dorsiventrality than do half of the Tristichaceæ. They have creeping roots which give rise to secondary dorsiventral shoots with large leaves developed in two ranks. The flowers are radially symmetrical, but are liable at times to become slightly dorsiventral by the abortion of some of the stamens. The inflorescence in Mourera is flat.

A further stage in this direction is shown in the South American Lophogynes and Apinagias, where the secondary shoots, while smaller in size, have become more or less flattened out and thalloid by the union of the bases of the leaves or the flattening of the stem. The flowers are usually markedly dorsiventral by the disappearance of the upper stamens. An extreme case of this construction is afforded by Castelnavia, where the (secondary) thalloid shoots lie flat upon the rock, like the (primary) shoots of Lawia in the other order.

Another section of the family is represented by the Podostemons, occurring in Asia and America, and probably in Africa also, where the root is more or less cylindrical and creeping, and gives rise to secondary shoots which are only slightly dorsiventral, chiefly in their branching, and which bear highly dorsiventral flowers. Allied to this genus, as well as perhaps to Lophogyne, etc., is Mniopsis, which is confined to South America, has a thallus like Lophogyne, and a flower like Podostemon.

Sphærothylax abyssinica Warming (Abyssinia) has what is apparently a primary axis that grows up to a considerable height, bearing compound leaves and flowers, while at the same time the root at the base forms a flat thallus, something like a lichen or a liverwort, bearing secondary shoots which remain very short, and ultimately become floriferous also. The flowers are highly dorsiventral. In *H. olivaceum* and other species of Hydrobryum (India and Ceylon) the primary axis is much reduced, only seldom bearing flowers, but the flat lichen-like thallus or root is large, and gives rise to great numbers of secondary shoots, which ultimately become floriferous, with very markedly dorsiventral flowers.

In Dicræa and Griffithella the root flattens out, sometimes in long thread- or ribbon-like forms, sometimes in more condensed shape, sometimes even forming a goblet-like organ. On the edges are borne the secondary shoots, which are very short, lengthening slightly at the end of the season, when they bear each a dorsiventral flower.

In Farmeria (India, Ceylon) the roots are creeping and flattened, as in Hydrobryum, but the flower carries the dorsiventrality to the last stage, showing it in the interior of the ovary, and in the embryo. In one species, *F. metzgerioides* Willis, the fruit contains only two seeds, and does not dehisce, the seeds germinating *in situ* on the rock. This is the only species in either family which has a proper arrangement for preventing its seeds from washing away.

In none of the species with flattened highly dorsiventral root does the stem or shoot of the secondary shoots show any sign of flattening out, while in none of those with flattened secondary shoot does the root show sign of flattening. The former are mainly characteristic of India and Ceylon, the latter of South America.

For the great variety in detail which all these plants exhibit reference must be made to the work of Warming* and to my previous work (*loc. cit.*) published upon the Indian and Ceylon forms. Tulasne's monograph† also contains a fine set of pictures of the families.

We may now proceed to deal in more detail with the various structural features of these families, which have generally been looked upon as adaptations. In the first place, let us take the flattened thallus-like roots or secondary shoots. These have been generally regarded as adaptations to withstand, or to avoid, the rush of the water. But, as already pointed out, the forms that are exposed to it have no marked "Zugfestigkeit." In my monograph of the Indian forms I expressed the opinion that the adaptation was rather to shallow water than to rushing water. The most highly adapted form, on either view, is *H. olivaceum*, and yet this very form, at the period when the rush of the water is most violent, possesses a tall primary axis with a great bunch of leaves at the end, an axis, moreover, which can hold fast if the flat creeping thallus be removed. Until I came to Brazil I was still in some doubt as to whether these dwarf forms and closely attached thalli might not be regarded as in some degree at any rate adaptations to rushing water, but what I have seen here has completely destroyed that idea, and enabled me to write this paper. Here there are no flat creeping root thalli, but instead there are, as in *M. Weddelliana*, flattened secondary shoots, forming thalloid outgrowths. Now these thalli, though with far less holdfast than the Indian root thalli, live in more rapid water. Never or very rarely, though for many years I have been familiar with the habitats of the eastern forms, have I seen any in such rapid or violent water as the forms which grow in the State of Rio de Janeiro. I have even found *Lophogyne arculifera* with the water falling on to it from a measurable height. Even the large Brazilian species of Mourera, Apinagia, and Marathrum live in very swift water without any difficulty, as may be very strikingly seen in Plate 62 of v. Wettstein's 'Vegetationsbilder aus

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* Warming, "Familien Podostemaceæ, I–VI," 'Kgl. Dansk. Vidensk. Selsk. Skr.,' 6 raekke, ii, 1881; ii, 1882; iv, 1888; vii, 1891; ix, 1899; xi, 1901.

† Tulasne, 'Monographia Podostemacearum," 'Arch. du Mus. d'Hist. Nat.,' vol. 6 (1852).

Südbrasilien.' The conditions of life are so absolutely uniform that any species can live in practically any place affected by these orders, though at any one locality they will more or less group themselves each in the kind of place that best suits it.

It therefore appears to me in the highest degree improbable that either primary root or secondary shoot thallus can be looked upon as an adaptation to violent water, especially when we consider that one form is mainly Asiatic, the other mainly American, while, further, they both share their habitats with the very slightly modified Tristichas, and with the also slightly modified Podostemons. As I have before stated, the adaptation, if adaptation there be, is to shallow, not rushing, water, but even upon that it is as well not to lay too much stress, because *T. hypnoides* and some species of Podostemon, neither of them with any thalloid flattening, are as well suited to, and grow in, the same shallow water as any of the thalloid forms.

The only thing that it is safe to say, after 40 years during which opinion has at first gone headlong in favour of adaptation and afterwards against it, is that the small forms can live in any depth of water in which the conditions of illumination will allow their life to go on, while the very large forms are only to be found in the larger rivers.

If all the extraordinary morphological differences between these plants were to be regarded as adaptational to the extremely small or non-existent differences in their conditions of life, the adaptation in land families living under more variable conditions would have to be something positively astounding. Or again if these differences are adaptational, why do we get one kind in one country, another in another, though there are similar species of the same genus living in both? Or why in one genus (Hydrobryum) do we get "root" thallus, in another (Lawia), living in the same place in the same river, "shoot" thallus?

To pass on now to the flower. In the Tristichaceæ, which are the less modified forms, it is simple and usually regular, some species of Tristicha itself having only one or two stamens on the lower side. In the Podostemaceæ it is sometimes regular, but more often dorsiventral, frequently to a very high degree. Now dorsiventrality has often been supposed to be an adaptation to insect visits; it is usually supposed only to be found in lateral flowers and in flowers which stand horizontally when open. But in the Podostemaceæ it is at its highest degree, and that the most extreme known in the higher plants, in flowers which are anemophilous, terminal, and erect. In my monograph of the Indian forms I have gone fully into this question, and may refer to that work for details. The conclusion there reached was that "the dorsiventrality of the flowers, which is the most important morphological character in the classification of the order, is a direct result of, or in direct correlation with, that of the vegetative organs, being greater the greater the dorsiventrality of the latter." And again "it seems then not unreasonable to conclude that the dorsiventrality of the floral organs has been, so to speak, forced upon them by that of the vegetative organs or by their position upon the latter without any reference to advantages or disadvantages to be derived from it in the performance of the functions of the floral organs themselves. The only demand made upon them, so to speak, has been that they should not cease to set seed."

A comparison of the characters used for separating the genera of Podostemaceæ proper, *e.g.* by Warming in Engler and Prantl's 'Natürlichen Pflanzenfamilien,' shows at once that practically all these characters, *e.g.*, suppression of the stamens of the upper side of the flower, obliquity of the gynœceum, mode of opening of the spathe, etc., are simply expressions of, and involved in, the degree of dorsiventrality of the flower. As I regard this, as above reiterated, as being due to a large extent, at any rate, to the vegetative dorsiventrality, I have consequently employed vegetative characters in addition to the floral in determining the Indian genera, and in the work which I am carrying out upon the South American forms I propose to do the same.

By no stretch of imagination can the dorsiventrality of the flowers be regarded as adaptational, and I have elsewhere shown good reason to suppose that in general it follows that of the vegetative organs. But in this paper we have seen reason to believe that that of the vegetative organs also is not adaptational. No gain whatever comes to these plants, in other words, from all their wonderful morphological changes, and the whole differentiation of the two orders into nearly 30 genera with numerous species is almost entirely an expression of the dorsiventrality forced upon them by their plagiotropic growth. The least dorsiventral and least modified species of the least modified genus of the Tristichaceæ can and do live in nearly all the places occupied by the two families, and the whole differentiation of the families is merely *de luxe*, and without any adaptational signification whatever.

Finally, let us consider the seed. In most of the plants of these orders there are very numerous (200–600) minute seeds, whose outer coat becomes mucilaginous when wetted, but in some the number is much reduced, especially in Farmeria, where in one species there is a dehiscent fruit with about four seeds, in the other, *F. metzgerioides* Willis, an indehiscent fruit with only two, which germinate *in situ*.

Now if these plants were really adapted to their habitat, one of the first things one would expect to find in them would be some arrangement to enable the seeds to cling to the rocks upon which they find themselves shed. But there is only one species in which this is the case, *F. metzgerioides*, mentioned above. All the rest have capsules which open in dry air and shed the seeds upon the rocks. When a shower of rain comes the mucilaginous layer of the seed swells, and as it dries it attaches the seed firmly to the rock. This has been looked upon as an adaptation for clinging to the rock by many people who have forgotten that when again wetted, as for instance by the rise of the water in the rainy season, the mucilage again softens and the seed washes away. The seeds once afloat have but a small chance of arriving at suitable growing places, for if dropped in quiet water they will not grow, and if carried to rocky places it is very unlikely that they will catch in anything to form a place for germination. Seedlings I found in Ceylon to be extremely rare, excepting only in *F. metzgerioides*. Only when a seed gets caught in a crack in a rock, or in a hole in the old thallus, does it get any chance to grow. As soon as it germinates it produces root hairs to fasten it to the substratum, but even these are often not produced quickly enough, and I have found, in trying to germinate seeds in the Botanic Gardens in Rio de Janeiro, that even when the cotyledons are open, and a number of root hairs attached to the rock, they may yet be washed away. My experience as yet with germination trials leads me to suppose that it takes at least from 500 to 1000 or more seeds to give three or four seedlings, and of these, perhaps, one may come to maturity. This being the case it is impossible to say that the order—except in the one species *F. metzgerioides*—shows any adaptation in this, one of the most essential things, one would think, in which it might, with its wealth of variety in structure, be adapted. Even in *F. metzgerioides* it is by no means certain that the indehiscent fruit is an adaptation. This form is the most highly dorsiventral of the whole family, and the peculiar feature of the indehiscent fruit may be merely another expression of the increasing dorsiventrality which runs throughout the Podostemaceæ.

After a consideration of all the features of these orders, then, we come to the conclusion that any and all of the adaptations that there may be in them are to be found in *T. ramosissima*, the least modified of all. As we pointed out at the commencement of the paper, in this species (and the same features appear throughout the two families) these features are—(1) a great and regular development of secondary shoots upon creeping, closely attached roots; (2) the development of haptera or clinging organs to attach the plants to the rock; and (3) the absence of intercellular spaces. Beyond these there are practically no characters in the orders that can possibly be regarded as adaptational, and most certainly not the characters—of thallus formations, dorsiventrality of flower, etc.—which really characterise the orders, and enable them to be separated in the natural system. But are even these characters which we have enumerated really adaptations ? The development of secondary shoots upon roots is by no means uncommon, though not usually carried to such a pitch of regularity and perfection as here. The absence of large intercellular spaces may be directly ancestral; if the orders came, as is quite possible, directly from plants growing upon the banks of the water, their members may never have had large intercellular spaces at all, these being quite useless in their mode of life. Haptera, perhaps, are really adaptations, as they are all but unique, and appear to be modified root branches, but they form the only feature in the two orders that one can point to that is at all probably an adaptation.

Geographical Distribution.—Another argument in favour of our main contention may be derived from the geographical distribution of the Tristichaceæ and Podostemaceæ. The genera which are widely distributed are Tristicha* in the one family and Podostemon† in the other, both of them genera which are comparatively little modified from the earlier types of the orders. Of the other genera the distribution is, roughly, the following :—

Lawia	W. India and Ceylon.
Weddellina	Guiana and N. Brazil.

Œnone......................	Guiana and N. Brazil.
Marathrum	Mexico to Brazil.
Rhyncholacis	Guiana.
Apinagia	Guiana and Brazil.
Lophogyne	Province of Rio de Janeiro.
Mourerera	Guiana, Brazil.
Lonchostephus	River Amazon.
Lacis	River Amazon.
Dicræa......................	Madagascar, India, Ceylon.
Hydrobryum	India, Ceylon.
Ceratolacis	Brazil.
Mniopsis	Brazil.
Oserya......................	Brazil to Mexico.
Castelnavia	Brazil.

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* Mexico, S. America, Africa, Madagascar, India.

† Ohio to Argentina, Ceylon, India, probably Africa.

Cladopus	Java.
Willisia	W. India, Burma.
Griffithella	W. India.
Farmeria	S. India, Ceylon.

The African species are as yet too little known to make it possible to give their distribution; the genera to which they really belong have yet to be made out in many cases, but are almost certainly all, or nearly all, different from the American and Indian.

In other words, the only widespread genera are the non-specialised ones, while the more specialised the genus, on the whole, the less is its area of distribution. The non-specialised forms live everywhere with the specialised, and are every whit as well suited to the positions, which show no differences in general conditions of life. This result may well be compared with that which I traced for the Dilleniaceæ* in a previous paper, being exactly parallel with that.

Or, again, take the well-known fact that in the Podostemaceæ the species are usually very local in distribution, as has frequently been pointed out.† The best known instance is Castelnavia, where in the same river, the Araguaya, some of the species differ at every cataract. Now, in the case of different species of a genus on land, it has been customary to say that they have been evolved to suit different mixtures of the conditions of life, and the absence of many species of one genus in most plants of still water has been put down to their uniformity of conditions. But here this explanation will not hold. The physical conditions of life at all cataracts in the Araguaya are the same, and there is no mixture with other forms of life at all. And this must have been true since the foundation of the family. And yet the Araguaya contains seven species of this one genus, a genus, moreover, which is almost confined to this river, in which there occur besides only one or two species of Oserya and Apinagia. It is another expression of the fact to which attention has often been called,‡ that isolation, as isolation, favours the production of new species. Why this should be so, we cannot at present say, but the fact remains. These species of the Araguaya are, of course, each endemic to its own few waterfalls, and to them may be applied the remark which I have already made* about the endemic species of Ceylon, that (p. 13) "in general they have characters which are, so far as one can conceive, useless in the struggle for existence; they occur in places where that struggle cannot have been very keen, or between very large numbers; they often occur alongside of their most nearly allied species, and very often the differences in character are such as can hardly conceivably have arisen by the selection of infinitesimal variations." The whole argument of that paper should be read in connection with this, as it produces evidence to the same end from a study of quite a different nature from that here dealt with.

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* Willis, "The Geographical Distribution of the Dilleniaceæ as illustrating the Treatment of this Subject on the Theory of Mutation," 'Ann. Perad.,' vol. 4, p. 69 (1907).

† Weddell, "Sur les Podostemacées en général et leur Distribution Geographique en particulier," 'Bull. Soc. Bot. France,' vol. 19, p. 50 (1873); Goebel, 'Pflanzenbiologischen Schilderungen,' vol. 2, pp. 331, 374; Willis, "Studies in the Morphology and Ecology of the Podostemaceæ of Ceylon and India," 'Ann. Perad.,' vol. 1, p. 450 (1902).

‡ Willis, "The Floras of Hilltops in Ceylon," 'Ann. Perad.,' vol. 4, p. 135 (1908).

Absence of Adaptation.—By a consideration of all the facts which have been brought forward, we are thus forced to the conclusion that as there are not, and have never been, any changed conditions to which these families have or had to be adapted, there cannot have been in them any adaptation to changed conditions after the first adaptation which enabled them to live on the rocks in running water. The whole of the extraordinary morphological changes through which they have gone are without any adaptational significance whatever. The conditions of life under which *T. ramosissima* exists are the same as those under which the rest of the families exist, and have always existed since the evolution of the families began. In spite of the great variety of form and structure, it is impossible to say that any one form is better suited to the conditions of life than any other. The most "primitive" forms, Tristicha and Podostemon, which have neither flattened shoot-thallus nor root-thallus (except in one species of Podostemon, which perhaps should form another genus) are the most widespread and the most common.

It may be objected, perhaps, that the modification and adaptation to the simple change of conditions involved by getting into the water is still going on, and is enough to account for all the modifications that the families have undergone. However well A may be adapted, it is of course obvious that B may be better adapted. But if it requires the evolution of two whole families with 30 genera and over 100 (perhaps over 200) species of the most various form to meet this need, we have a very remarkable case before us, when we consider that the first founders of the families must have been adapted to life in running water, or the families could never have commenced. If this were to be the case, we should expect enormously greater variety than actually exists among the other plants of the vegetable kingdom.

As has been pointed out, the most obvious feature in these plants where, if anywhere, we should expect adaptation, viz., the attachment of the seeds to the rocks, shows none, except in one species only, and that the most highly modified species in the whole family. Unless the haptera be an adaptation, this is the only "adaptation" in the whole group, and if it takes all this amount of evolution to get to one adaptation, there cannot be much selection of advantageous variations. The morphological changes in these plants are so large and striking that if there were any adaptation involved in them, there would surely be some evidence to prove it, but there is none, and in all my work on these plants I have not knowingly suppressed any evidence one way or the other, or failed to put down anything that I have observed.

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* Willis, "Some Evidence against the Theory of the Origin of Species by Natural Selection of Infinitesimal Variations, and in Favour of Origin by Mutation," 'Ann. Perad.,' vol. 4, p. 1 (1907).

Not only so, but the forms that are most like the common ancestors, and possess none of the thalloid structures of the more bizarre genera, viz., Tristicha and Podostemon, are much the most widespread and the most common. As with the Dilleniaceæ and other orders instanced in my previous papers, it is much simpler to regard these as parent genera which have split off the others by mutation in different places. Everywhere they live together with the more complex and modified forms, and in equal abundance. None of the latter have one quarter of their range.

Again, the more complex forms have flowers which are modified in a direction that can only be looked upon as disadvantageous, if anything. I have already gone into this question in another paper, and need only refer to it here. By no conceivable advantages could the flowers have been the subject of natural selection.

There is no adaptational need for the complex forms; if they are better suited to the general conditions of life of the orders, there is nothing to prove it, and they are not a conspicuous success, except in places that happen to prove exactly right for them, as for instance *Lawia zeylanica* in the shallow streams of the Bombay Ghats. The evidence of the local endemic forms is also against this view of still-progressing adaptation.

The conditions of life, as has been pointed out, are as nearly as possible absolutely uniform, much more so than in other water plants, which have a certain amount of soil differences, competition with plants of other families, and so on, to face. So great is the destruction of seed before germination in these families that until quite late in the season there is usually ample space upon the rocks, so that there can be but little competition between them for space. Competition for food cannot exist in water running so rapidly.

The only "adaptation" that can be conceived of as going on in these plants under these circumstances is to the ever-present force of plagiotropism, but if this be admitted, it is also admitted that there can be definite evolution-factors, which it is one of the objects of this paper to demonstrate. Adaptation by natural selection to this force, with the enormous destruction of seed that goes on, is almost inconceivable, however.

The Process of Evolution.—It follows from the above that, as there is no adaptational selection, or selection of characters better suited to the struggle for existence, the process of evolution in these families from the simpler to the more complex forms cannot have been by the gradual accumulation of infinitesimal variations, unless these can also be selected by the one permanent cause acting upon the families, viz., their plagiotropism. Many of their variations, too, are not dorsiventral, and plagiotropic life would not be likely to select them. The more complex and modified forms are in no way whatever superior to the simpler forms; both live together, and one shows no sign of exterminating the other. It is all but impossible, however, to imagine that plagiotropic life could select infinitesimal variations, and as I have shown in dealing with the endemics of Ceylon that mutation is the only possible explanation of them, so here I propose that we accept the theory of mutation as the only feasible explanation at present possible of the facts with which we have to deal. Mutations not being liable to go back, it is possible to accumulate them, unless any one of them should prove of very serious disadvantage, in which case it would be eliminated by natural selection.

Taking the evidence which is here brought forward with that which I have set forth in other papers already quoted, added to the fundamental work of de Vries, it seems to me that a good case is now fully made out for mutation, and that the onus of proof is completely thrown upon the infinitesimal variationists, while a very fair case is made out for mutation without natural selection. Analogy of the conditions and phenomena which we have been considering in this paper with those which occur throughout the vegetable kingdom lead almost irresistibly to the conclusion that mutation without natural selection must be a theory of general applicability, although there is no reason to exclude natural selection from operation upon a smaller scale than that for which it has hitherto received credit—chiefly, be it remarked, it operates by destroying disadvantageous variations, unless they are compensated by advantageous ones at the same time. This subject, however, leads into great issues, which must be left for subsequent consideration. A *résumé* of the arguments which have led me to take up the position I hold in regard to this question is given on p. 208* of the third edition of my 'Manual and Dictionary of the Flowering Plants and Ferns, 1908.'

The Mechanism of the Process of Evolution.—So far the reasoning has been straightforward, and we have come to the conclusion that there must have been evolution by means of mutations, and without natural selection in the ordinary meaning of the term. When we come to consider what this really means, we are met by great difficulties.

There is one, and apparently only one, or at most two, permanent deflecting factors, that act now, and have always acted since the evolution of the families began, when their first ancestors took to life in running water. These are, first and foremost, their plagiotropic method of growth, forced upon them by the fact that they live only upon an unyielding substratum; they have not, and can never have had, primary roots going downwards into the rock, and are thus, one might almost say, cut in half, or deprived of one-half of their polarity. The other factor is the longitudinal strain of the water, which to a large extent acts in the same general direction.

Now there is also one, and apparently only one, or at most two, permanent results visible in the evolution of the members of these families, viz., the plagiotropic or dorsiventral habit both of the growing organs and the structure of the flower, which is by far the most striking feature in them; added to this is the considerable elongation and division of the leaves (or root-thalli) in some of the forms. Their differentiation into genera and species depends to a large extent upon these two features, which are merely expressions of increasing dorsiventrality, and increasing size and division of leaves (or root-thalli).

Now, when one sees in a family where the complexity of the ordinary evolution process is so much simplified as we have seen it to be here, one principal and one subsidiary deflecting factor, and one principal and one subsidiary result, it is very difficult to avoid the conclusion that these are cause and effect. But it must be again insisted upon, and clearly understood, that there has been no selection of advantageous variations. All the forms, whether slightly or highly dorsiventral, can live in practically all the localities affected by the families, so that the increased dorsiventrality of the more modified forms is of no advantage to its possessors. The least modified forms are the most common and widespread (and this by the way appears to be the general rule in other families, as I have already pointed out), while the much modified ones are local.

This being so, we are almost forced to believe that a definite deflecting factor can make evolution go more or less in a definite direction, without regard to any advantages to be gained by it, though any modification that was seriously disadvantageous would be weeded out by natural selection.

But now comes a serious difficulty. If we have to admit that evolution can take place without natural selection, and that there is no selection of advantageous variations, why do we (so far as present evidence goes) not get growing together all the stages in the evolution of the very numerous species of these families? And why are there any gaps between the species? Why are they not filled up by intermediate forms, only differing from one another in very slight degree? (If gaps once arise between species, larger ones will inevitably, so far as one can see, arise between genera, so that we may leave the genera out of account.) Does this mean that mutations may be larger than we usually imagine? Or does it mean that a mutation in one direction involves further mutation in the same direction? Or what does it mean?

It would scarcely seem, perhaps, as if the actual direction of mutation could have been selected by the forces acting on these plants, unless in the progenitors there appeared several mutations in different directions involving others in the same directions, all of which were selected.

As one can almost say that no variations other than dorsiventral ones or variations in the direction of longer or more divided leaves or thalli, have been perpetuated, and as the dorsiventrality is no advantage, and as there is no natural selection except in the direction of extermination, it is very difficult to escape the conclusion that the evolution was more or less guided in a definite direction by the plagiotropism. Perhaps this force or natural selection exterminated variations, other than slight, in other directions.

On the whole, we are inclined to think, though as yet with great diffidence, and with an open mind, that the evolution of these families was by indiscriminate mutation, or mutation in every direction, without natural selection, the mutations in the direction of dorsiventrality and perhaps in the direction of longer and more divided leaves being on the whole more easily perpetuated than others—many of which would be killed out by natural selection—and this on account of the permanent deflecting forces acting on these plants, and which we may perhaps call their evolution-factors. A strongly marked evolution-factor, like plagiotropism in these families, can compel evolution to move on the whole in a definite direction, without any reference to the advantages or disadvantages to be derived from so moving.

To accept this result as general will explain without any difficulty the presence of such countless numbers of useless characters in plants,* and may help to account for the great changes in the botanical landscape which seem often to accompany the greater changes in the geological landscape—the great change of conditions on getting into plagiotropic water life seems to have produced the great variety in the plants we have been considering, and perhaps the greater landscape changes were similarly accompanied by great changes of conditions.

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* *Cf.* De Vries, 'The Mutation Theory,' vol. 1, p. 208 (Delbœuf), 1912.

The Action of Certain Drugs on the Isolated Human Uterus.

By James A. Gunn.

(Communicated by Prof. C. S. Sherrington, F.R.S. Received March 7,—Read April 30, 1914.)

(From the Pharmacological Laboratory, Oxford.)

I have elsewhere shown* that, in experiments on the isolated mammalian heart, it is perfectly possible to keep the exsected heart in cold Locke's solution at ordinary room temperatures for hours and still to obtain powerful and regular contractions of the heart when, after this procedure, it is subsequently perfused with warm oxygenated Locke's solution in the usual way. Similar observations have been made on other contractile tissues and will be dealt with in another communication.

In the meantime, it is sufficient to point out that those observations open an easy way to experiments on a certain number of isolated human tissues, removed for surgical reasons, by which experiments certain questions can be answered which cannot readily, if at all, be decided in any other way.

So far as I am aware, this is the first time that pharmacological experiments of this nature on isolated human tissues have been performed, and by the simplification of technique dependent upon those observations on the survival of involuntary muscle at ordinary temperatures, a field is open for exact quantitative pharmacological experiments immediately upon those tissues, whose reaction to drugs it is the final aim of pharmacology to determine. These experiments can be made under similar conditions to, and therefore entirely comparable with, experiments made on tissues of those mammals ordinarily used for pharmacological investigation.

One of the questions which require to be answered has regard to the nature of the sympathetic innervation of the human uterus and its response to certain drugs.

It has been shown by Langley and Anderson† that the sympathetic nerve supply to the uterus of the rabbit is motor in quality, whether the uterus is in the pregnant or non-pregnant condition, and that adrenine has a similar motor effect on it. On the other hand, it was discovered independently by Cushny,‡ Dale§, and Kehrer‖ that the uterus of the cat responds to sympathetic stimulation or to adrenine by a motor effect when pregnant, but by an inhibitor effect when non-pregnant; in the latter case, therefore, differing from the response of the rabbit's uterus.

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* Gunn, 'Journ. of Physiol.,' vol. 46, p. 508 (1913).

† Langley and Anderson, 'Journ. of Physiol.,' vol. 19, p. 122 (1895); Langley, *ibid.*, vol. 27, p. 252 (1901).

‡ Cushny, 'Journ. of Physiol.,' vol. 35, p. 1 (1906).

§ Dale, 'Journ. of Physiol.,' vol. 34, p. 163 (1906).

‖ Kehrer, 'Arch. für Gynäkol.,' vol. 81, p. 160 (1906).

It has recently been shown* that a still different type of sympathetic innervation holds good for the uterus of the rat and the guinea-pig, for in those animals adrenine inhibits the uterine contractions both when the uterus is pregnant as well as when non-pregnant.

There are, therefore, three known types of predominant sympathetic innervation of the uterus in different species of animals, as shown in the following table:—

	Reaction to Adrenine.	
	Non-pregnant uterus.	Pregnant uterus.
Rabbit	Motor.	Motor.
Cat	Inhibitor.	Motor.
Guinea-pig	Inhibitor.	Inhibitor.

Now the type of innervation in the pregnant uterus of the rat and guinea-pig raises the important question, namely, what is the quality of the sympathetic innervation of the human uterus? Does it resemble that of the rabbit, that of the cat, or that of the guinea-pig? This is obviously a question of supreme importance in the use of adrenine or other sympathomimetic substances in human labour, because if the pregnant human uterus is to be relaxed by those drugs (as is the pregnant uterus of the rat or guinea-pig) then the employment of them in inertia uteri or in post-partum hæmorrhage is not only valueless but definitely dangerous.

Though the kindness of Drs. Whitelocke and Dodds-Parker, Surgeons to the Radcliffe Infirmary, Oxford, I have been able to obtain sufficient material to satisfy at least part of this inquiry. The former gave me the uterus and a Fallopian tube from one case, and Fallopian tubes from two other cases; and the latter a Fallopian tube from one case. The organs were removed from surgical necessities. As soon as they were removed, they were put into cold Locke's solution and conveyed to the laboratory. There they were put into a bath of Locke's solution, oxygenated and at a temperature of 37°, and the movements recorded, the method employed being the now familiar method used for isolated mammalian organs and first used for the uterus by Kehrer.

The isolated human Fallopian tube, when put into warm oxygenated saline solution, almost immediately executes rhythmical movements, not in any decided way dissimilar from the contractions of the isolated uteri of ordinary experimental mammals under similar conditions of experiment. Indeed the readiness with which the human Fallopian tube passes into rhythmical contraction makes it clear to me, after experience of other rhythmically contractile tissues under the same conditions, that the Fallopian tube possesses a high degree of spontaneous rhythmicity.

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* Gunn and Gunn, 'Journ. of Pharmacol.' (1914).

Several experiments which have been made have shown conclusively that adrenine has a powerful motor effect on the human Fallopian tube.

The effect of adrenine is shown in fig. 1; in this case it produced a rise of tonus with conversion of slower rhythmic contractions into more rapid smaller ones.

Fig. 2 is shown because it illustrates (1) what I have found in four experiments, the somewhat surprising fact that pituitrin has no pronounced effect on the human Fallopian tube; (2) because it shows the continued vitality of the organ after it had remained for 30 hours in cold Locke's solution, and the still normal response to epinine.

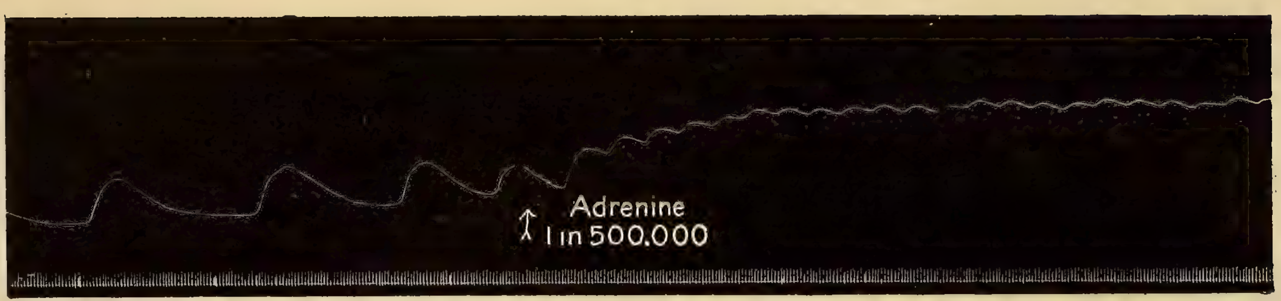
That the vitality of the uterus is great under certain conditions of keeping is a fact of which I was previously aware, because from unpublished experiments made in this laboratory in conjunction with Dr. Hudston, it was found that, after the guinea-pig's uterus had been kept in Locke's solution, at temperatures of from 3 to 7° C., it still may execute rhythmic movements when placed in warm oxygenated Locke's solution, after having remained quiescent at the low temperature for as long a period as seven days.

In regard to the uterus proper I have as yet obtained only one for experiment. This uterus was removed from a patient, non-pregnant and about 40 years of age. The uterus was removed for disease of one Fallopian tube along with a partial fibroid condition of the uterus itself. The other Fallopian tube and part of the uterus was apparently healthy. The latter was cut into strips and tested in the usual way.

The rhythmic movements of the uterine strips were much slower and more infrequent than those of the Fallopian tubes.

Fig. 3 shows the effect of adrenine 1 in 250,000 on a strip of uterine muscle. The strip had shown contractions lasting from about 30 to 60 seconds at intervals of from 3 to 5 minutes. At the end of one of those contractions adrenine was added to the bath (fig. 3). This produced a strong tonic contraction with production of superimposed smaller waves. Other strips which were tested gave a similar result.

It was interesting to compare, for further guidance, the effect of the same concentration of adrenine on the Fallopian tube belonging to the same uterus. Fig. 4 shows the result obtained.



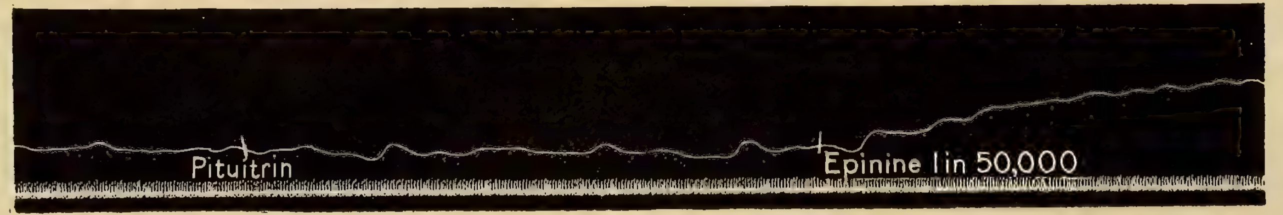


FIG. 1.—Isolated Human Fallopian Tube. Showing Motor Effect of Adrenine. Contraction recorded upwards in this and following tracings.

FIG. 2.—Isolated Human Fallopian Tube, 30 hours after removal. Showing Negative Effect of Pituitrin and Motor Effect of Epinine.

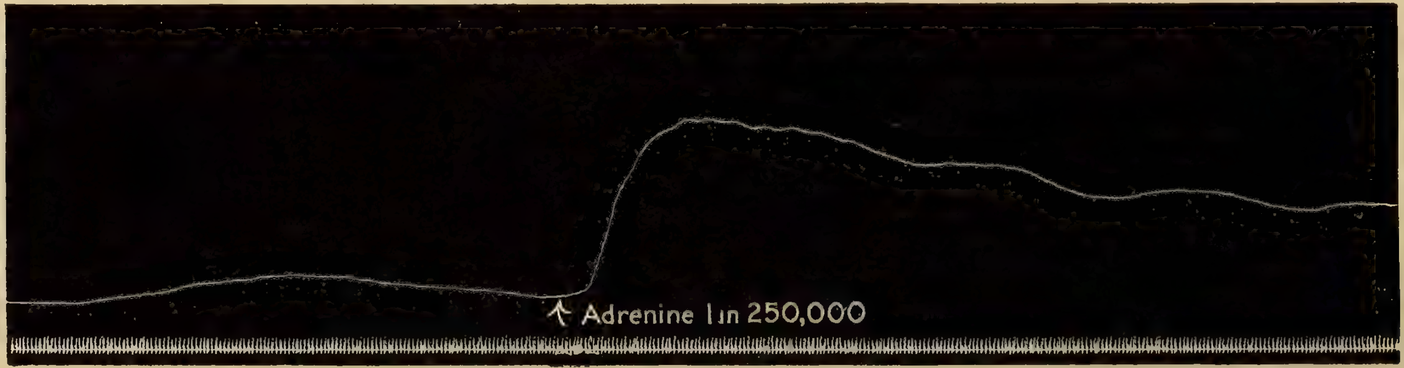


FIG. 3.—Isolated Strip of Human Non-pregnant Uterus. Showing Motor Effect of Adrenine.

It is clear from these experiments that adrenine has a powerful motor action on the non-pregnant human uterus and Fallopian tube. The presumption is, therefore, that the sympathetic nerve supply to it is motor in quality.

Pituitrin decidedly stimulates the contraction of a strip of human uterus as shown in Fig. 5. The effect is produced much less promptly than the effect of adrenine. No such pronounced effect could be obtained on the Fallopian tube belonging to the same uterus, the addition first of pituitrin 1 in 800 and then 1 in 400 having no effect in 15 minutes, whereas the subsequent addition of adrenine 1 in 250,000 produced immediately powerful and enduring contractions. The negative effect of pituitrin on the tube is an unexpected result, seeing that the musculature of the tube is continuous with that of the uterus proper. It suggests that deductions cannot be drawn from experiments on the Fallopian tube alone to embrace the uterus proper.

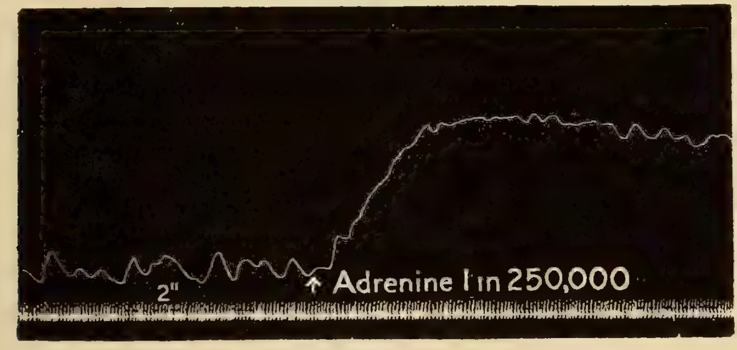


Fig. 4.—Isolated Human Fallopian Tube from same Uterus used in fig. 3.

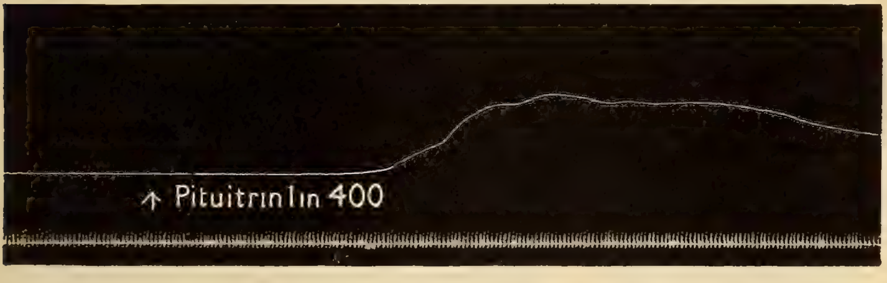


Fig. 5.—Isolated Strip of Human Non-pregnant Uterus. Showing Motor Effect of Pituitrin.

It has not been possible yet to secure for experiment a pregnant human uterus, which is of course rarely removed. Unless, however, the human uterus has a still different type of sympathetic innervation from that of all the animals which have been examined, the probability is that adrenine will have a motor effect on the pregnant human uterus, and that, therefore, the use of sympathomimetic substances in labour is justified.

Summary.

The movements have been recorded of the isolated non-pregnant human uterus and Fallopian tube. Adrenine has a powerful motor action on both these organs. The deduction is drawn that this is the qualitative effect of sympathetic innervation of the human uterus, at least when non-pregnant.

Pituitrin also stimulates the human uterus proper to contraction, but no such effect has been definitely obtained on the Fallopian tube.

The Presence of Inorganic Iron Compounds in the Chloroplasts of the Green Cells of Plants, considered in Relationship to Natural Photo-synthesis and the Origin of Life.

By Benjamin Moore, D.Sc., F.R.S., Professor of Biochemistry, University of Liverpool.

(Received March 11,—Read April 30, 1914.)

(From the Johnston Biochemical Laboratory, University of Liverpool.)

It has been demonstrated by Moore and Webster* that colloidal solutions, or suspensions, of salts or oxides of iron, in presence of dissolved carbon dioxide and with the energy supply of sunlight, possess the power of synthesising formaldehyde. Since this is known to be the first step in the process of organic synthesis of the substance of all living plants and animals from inorganic material and must, moreover, have occurred in past ages over immense areas of the earth's surface before life began to exist on the planet, the conclusion was drawn that life must have originated by continual development of more and more complex organic substances from this simple commencement.

It is in this first stage of all that the greatest production of chemical energy occurs, and accordingly a transformer of light energy into chemical energy is essential. Although the more highly organised carbohydrates and proteins still require catalysts for their synthesis, weight for weight they contain scarcely any greater storage of chemical energy than formaldehyde, and once an accumulation of organic material has been reached, even the fats and their allies can easily be synthesised by the combination of linked exothermic and endothermic reactions by the living cell acting as a transformer, without the use of external energy such as that of sunlight.*

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* 'Roy. Soc. Proc.,' B, vol. 87, p. 163 (1913).

Once organic matter has been synthesised, the living cell can oxidise one portion of this to reduce still more another portion. In this manner the animal cell can oxidise carbohydrate, for example, and use the energy so set free to build up another portion of carbohydrate into fat which weight for weight contains double as much chemical energy as the carbohydrate, without in the process making use of an external source of energy.

At the commencement, however, when only water and carbon dioxide are the available materials, it is indispensable that an external source of energy such as sunlight should be available, and a suitable mechanism, or chemical system, for the transformation of this store of energy into the chemical energy of organic compounds.

Such a transformer has been recognised for a long period in the chloroplast or chlorophyll-granule of the green cell of the higher plant.

Since the days of de Saussure,† now over a century ago, the green colouring matter of the leaf, chlorophyll, has been regarded as the fundamental agent for this world-wide photo-synthesis. But it is remarkable how completely this view is based upon indirect or circumstantial evidence, and how little, if any, direct observation can be cited in its support.

Chlorophyll is known by the biochemist to be one of the most complex of substances, comparable to hæmoglobin in its molecular structure, and yielding a host of disintegration constituents themselves complicated substances of high molecular weight. Between the simple colloidal molecules of inorganic iron salts in solution or suspension and such a highly complex organic substance as chlorophyll there is a wide hiatus, and it was with the view of discovering some intermediate links or finding some explanation for the gap that the present experiments were commenced.

Before describing the experiments in detail it is desirable to touch upon present views as to photo-synthesis in the green cell as far as these bear upon our investigation, in order to give an appropriate setting to the new facts, and show how present knowledge regarding the absolute necessity for the presence of iron in the green leaf, which has been hitherto devoid of all explanation, led up to these experiments.

Photo-synthesis with production of oxygen only occurs in the chlorophyll-containing parts of the plant, and only in these when they are exposed to light. Also, when a plant is allowed to grow in darkness, the leaves are found to be pale-yellow in colour, or chlorotic, instead of green. When a plant grown in darkness, and, as a result, possessing chlorotic leaves, is then exposed to light, the pale-yellow colour is rapidly replaced by a green, and then photo-synthesis is readily demonstrable by the evolution of oxygen and the appearance of starch granules.

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* See Moore, 'Recent Advances in Physiology and Biochemistry,' edited by Leonard Hill, pp. 135, 138, Arnold, London (1906).

† "Recherches Chimiques sur la Végétation" (1804), 'Ostwald's Klassiker,' Nos. 15–16

The above reasoning constitutes the whole of the evidence that chlorophyll is the primary cause in the first act of photo-synthesis. It is to be observed that the entire chain of evidence is inferential, and that in order to form a valid proof, chlorophyll would require to be the only substance present in the chloroplast, which is very far from being the case. No observer has ever obtained an appreciable and satisfactory synthesis with pure chlorophyll in solution or suspension when removed from the other constituents of the chloroplast. Certain observers have observed minute traces of formaldehyde formation with chlorophyll solutions or emulsions, but even these traces of photo-synthesis have been stoutly denied by other competent observers. In any case, the photo-synthetic effect produced is infinitesimally small compared to that observed in the intact green cell.

The most recent and careful experiments upon this subject are those performed by Usher and Priestley* and by Schryver.† Usher and Priestley found that when a chlorophyll-containing extract from green leaves was spread out as a film or emulsion on a gelatine plate, small, but distinctly demonstrable amounts of formaldehyde were formed on exposure to sunlight. But in this case there is gelatine and the inorganic colloids it contains shown by its ash to be present, and in the chlorophyll extract there would undoubtedly be iron salts present, because about one-fourth of the iron of green leaves comes away in the alcoholic extract.

Schryver worked with an ethereal solution of chlorophyll allowed to evaporate at room temperature on a strip of glass, and found that although such films of chlorophyll on glass produced no formaldehyde in darkness even in presence of moist carbon dioxide, a minute amount of formaldehyde was formed when the film was exposed to sunlight even in absence of carbon dioxide, and a distinct reaction when the film was exposed to sunlight in presence of moist carbon dioxide. The amount of formaldehyde formed in all such experiments is, however, very minute compared to the products of photo-synthesis under natural conditions by the complete chloroplast.

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* 'Roy. Soc. Proc.,' B, vol. 77, p. 369 (1906); B, vol. 78, p. 318 (1906); B, vol. 84, p. 101, (1911).

† 'Roy. Soc. Proc.,' B, vol. 82, p. 226 (1910).

Now the chloroplast contains a great deal more than chlorophyll, and when all the chlorophyll has been removed by some such reagent as hot alcohol there remains behind a colourless body, the so-called stroma. The chloroplast after the extraction is still a solid looking body, and to all appearances the only thing that has happened is that a thin layer of green colouring matter has been removed. There is no shrinking or shrivelling up of the chloroplast.

There is accordingly no experimental evidence that the primary agent in the photo-synthesis may not be contained in the colourless part of the chloroplast, and the chlorophyll may be evolved at a later stage in synthetic operations induced by some constituent of the colourless part. The function of the chlorophyll may be a protective one to the chloroplast when exposed to light, it may be a light screen as has been suggested by Pringsheim, or it may be concerned in condensations and polymerisations subsequent to the first act of synthesis with production of formaldehyde.

All these views and others are possible, and the function of chlorophyll in the chloroplast remains for solution, but it has not been proved that chlorophyll is the primary causative agent in the photo-synthetic process where the chief energy uptake occurs with formation of formaldehyde.

There are other pieces of experimental evidence apart from the repeated failures to obtain satisfactory synthesis with isolated chlorophyll which go to indicate that chlorophyll is not the transformer in the first link of the synthetic chain.

In the first place chlorophyll itself is a product of photo-synthesis, and therefore there must be some active photo-synthetic substance present in the chloroplast before the chlorophyll appears which indeed first produces the chlorophyll by its activity.

When a yellow etiolated leaf taken from the darkness is exposed to the light it contains no chlorophyll, but photo-synthesis, in the absence of chlorophyll, sets in, and chlorophyll itself is one of the products, not the originator or agent, of this photo-synthesis. The period from first exposure to light to the appearance of chlorophyll is too short to determine whether oxygen production and starch formation commence before chlorophyll is formed.

In the next place Engelmann,* by the application of his ingenious method of the oxyphile bacteria has clearly demonstrated two important facts; first, that the chloroplast alone, even when displaced from the rest of the cell, can, in presence of light, go on synthesising and producing oxygen;

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* 'Botanische Zeitung,' 1881, p. 446, and 1887, pp. 394, 410, 418, 426, 442, 458.

and, in the second place, a still more important point in our chain of evidence, namely, that certain leaves such as those of the yellow variety of elder, which do not produce chlorophyll when exposed to light but contain yellow chromatoplasts, cause synthesis and produce oxygen. These observations as to synthesis by healthy yellow leaves have been confirmed by other observers such as Tammes, Josopait, and Kohl.*

The strongest piece of evidence, however, that iron salts are more fundamental to photo-synthesis and take an earlier share in it than chlorophyll, is that furnished by that process frequently occurring in green leaves known as "chlorosis."

Chlorosis is a pathological condition of green leaves of considerable practical importance in arboriculture, and the discovery of its cause is, as Molisch states, one of the most interesting and beautiful in the history of plant physiology.

It was shown in 1843 by Eusebe Gris† that chlorosis naturally occurring in the leaves of shrubs or trees could be entirely removed either by applying dilute solutions of iron salts to the roots, or by placing the detached chlorotic branch in a dilute solution of iron, or even by painting the chlorotic leaf with a very dilute solution of an iron salt. In some cases within 24 hours, and in nearly all cases in a period of a week to 10 days, the green colouring matter developed in the leaves where none had been before.

These results have been often confirmed and have been extended by Salm Horstmar, A. Gris, and Sachs.‡ Molisch§ has, moreover, shown in a long series of experiments with different species of plants that all green plants, *even when fully exposed to light*, become afflicted with chlorosis and fail to develop chlorophyll when they are grown in a culture fluid especially made devoid of iron. As soon as the reserve store of iron always contained in the seed embryo and cotyledons has been exhausted in the primordial leaves, only chlorotic pale-yellow leaves are formed. These pale-yellow leaves rapidly turn green if minute quantities of an iron salt are added to the culture fluid, or even if the surface of the leaf be painted over with a dilute solution of an iron salt, as had been previously shown by Gris to be the case with pathologically chlorotic leaves. So that iron is as indispensable to the green leaf as it is to the red blood-corpuscle.

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* Quoted by Czapek, 'Biochemie der Pflanzen,' vol. 1, p. 447.

† 'De l'Action des Composés Ferrugineaux sur la Végétation,' 1843. See also 'Comptes Rendus,' vol. 19, p. 1118 (1844); vol. 21, p. 1386 (1845); vol. 23, p. 53 (1846); and vol. 25, p. 276 (1847).

‡ Salm Horstmar, 'Versuche über die Ernährung der Pflanzen,' 1856; A. Gris, 'Annales d. Scien. Nat.,' Series IV, vol. 7, p. 201 (1857); Sachs, 'Flora,' 1862.

§ Molisch, 'Die Pflanzen in ihren Beziehungen zum Eisen,' Jena, G. Fischer (1892). Many of the references given are quoted from this source.

The remarkable thing, in view of this failure to develop chlorophyll in absence of iron, is that chlorophyll itself is shown by all the more recent researchers to be quite free from iron.* Chlorosis and its cure by iron salts has accordingly remained a puzzle to plant physiologists ever since the time of the discovery of Gris.† The experiments to be recorded below furnish, for the first time, a rational explanation of chlorosis and its cure. The iron salts are necessary for the formation of the colourless portion of the chloroplast, for when all the chlorophyll and other fatty bodies and pigments are removed from the chloroplast by extraction with alcohol, and the colourless chloroplastic residue is treated with the micro-chemical tests for inorganic iron, a positive reaction in unmistakable degree is usually given by the colourless residue of the chloroplast.

This inorganic iron in presence of sunlight must give rise to photosynthesis and production of formaldehyde which is then carried on into sugar and starches by other constituents of the chloroplast, and it is probably here, somewhere in the later processes, that the chlorophyll finds its function. The chlorophyll itself, as shown by the facts of chlorosis, its removal by administration of iron, and the presence of iron salts in the colourless part of the chloroplast, is a product of synthesis from colourless substances or from the light-yellow pigment. For the production of the chlorophyll under normal conditions, both the presence of iron and the energy of sunlight are essential.

The reason for the earlier erroneous view that the chlorophyll molecule contained iron was that a certain fraction of the iron compounds contained in the green leaf becomes extracted by the alcohol used in the first extraction of the leaf,‡ so that all crude chlorophyll extracts contain iron. This, however, disappears on treating the alcoholic extract with benzol, and the product of purer chlorophyll separating from the benzol fraction is iron-free. At the same time its spectrum and other physical properties prove it to be unaltered chlorophyll.

Other facts which show the importance of iron compounds in the green leaf are that leaves which are not deciduous annually, such as pine needles, contain more iron in their later years, and also in leaves of annual growth the older the leaf is the more iron does it contain in its ash. Thus Boussingault found in the ash of young leaves of *Brassica* 2 per cent. of Fe_2O_3, while old leaves contained in their ash 9·64 per cent. *Lactuca sativa* had in the young leaves 2·67, and in the old leaves 6·43 per cent. of Fe_2O_3 in the ash. Another point is the curious conservation, resembling that seen in the animal economy, of the iron of the leaf in the case of deciduous leaves. Before the leaf drops a good deal of the iron is re-absorbed and stored for future use. This is shown by analyses of the iron of the leaves of *Fagus sylvatica* made by Rissmüller* in successive months. The figures quoted give quantities of Fe_2O_3 in 100 parts of dried leaves collected at the times of year stated—

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* See Molisch, *loc. cit.*, and R. Willstätter u. A. Stoll, "Untersuchungen über Chlorophyll,' Berlin, J. Springer (1913).

† See Czapek, 'Biochemie der Pflanzen.'

‡ According to early observations of Boussingault (Agronomie,' vol. 5, p. 128) from one-quarter to one-third of the iron is removed by the alcohol.

	May.	June.	July.	Aug.	Sept.	Oct.	Nov.
Oxide of iron.........	0·35	0·51	0·58	0·75	1·03	0·60	0·59

The gradual increase of iron content to a maximum followed by a fall as the leaves grow sere is very interesting.

It has been shown by Molisch (*loc. cit.*) that iron is an essential constituent for the growth of all plants, whether green or otherwise, but the saprophytic and parasitic plants which contain no chlorophyll require much less iron and, as a rule, contain much less in their ash. Our own experiments show that the histo-chemical reactions for iron develop much more slowly in the fungi and are much less intense in degree. These feebler reactions probably arise from organic compounds of iron slowly being decomposed in traces and setting free ionic iron. These organic iron compounds of the fungi are concerned with some other function than photo-synthesis or chlorophyll formation; they probably take a part in nuclear structures, for many nucleins are iron-containing, and, as has been shown by Macallum, after treating with acid alcohol to unmask the iron previously present in an organic form, the chromatin of nuclei always contains iron.

The reactions for inorganic iron are shown most markedly with the more lowly organised plants such as unicellular green plants occurring alone or in lichens, or in delicate algal threads, but when proper precautions are taken they can also be clearly demonstrated in the chloroplasts of the higher plants. The reactions are particularly well shown by the chloroplasts of aquatic plants, where, as is well known, the percentage of iron in the ash is also high.

These facts are in keeping with the natural order of evolution and are also in accord with other observations. For example, many algæ (such as certain Confervæ and Cladophora) deposit around them a layer of yellow to rust-red colour consisting of mixed ferrous and ferric oxides; this is often actively secreted from waters containing only traces of iron.

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* "Ueber die Stoffwanderung in der Pflanzen," 'Landw. Versuchsstationen,' 1874 ('Just's Jahresbericht,' vol. 2, p. 849, 1874).

A considerable number of lichens also secrete incrustations of the mixed oxides of iron to such an extent as to change their appearance to an iron-oxide or ochre colour, so that they have been termed by systematic botanists "formæ oxydatæ, ochraceæ" or "iron lichens." The iron-oxide forms a fine incrustation usually on the mycelium of the fungus. No association of this iron-oxide with a photo-synthetic function has ever been suggested, but in view of our present knowledge of the photo-synthetic activity of iron salts some investigation in this direction is highly desirable. It is an interesting observation of Molisch, from our point of view, that these "iron lichens" flourish exclusively on the oldest primitive rock-formations ("Urgestein"). They are never found upon chalk formations, but grow upon granite, gneiss, syenite, and porphyry. Molisch was unable to find inorganic iron in the other lichens, but this doubtless arose from the less delicate methods he had at his command at that time, and from the fact that the fatty bodies contained in the green cells of the alga of the lichen had not been removed. When the lichen is extracted with alcohol and Macallum's hæmatoxylin test then applied, the algal cells rapidly stain a deep blue-black, showing the presence of inorganic iron, while the hyphæ of the fungus only take on a brownish tinge during the same time, and only give a faint positive reaction at the end of some days or weeks.

It is somewhat remarkable that the presence of iron in the chloroplast should for so long have escaped discovery. The explanation probably lies in the fact that little attention has been given to the application to the green cell of the histo-chemical tests for iron since the discovery by Macallum of the more delicate hæmatoxylin iron test, as also to the delicacy of the chloroplasts to the more drastic earlier method used by Molisch, and to these factors may be added the difficulty with which some of the chemical reagents for iron penetrate the green cell, and the presence in the chloroplast itself of fatty and lipoidal substances which prevent the ingress of the water-soluble stains.

Macallum* in 1894 before his discovery of unmordaunted hæmatoxylin as a reagent for iron, and using then ammonium sulphide in glycerine as a reagent, states that bacteria gave no evidence of an organic iron compound, but in the Cyanophyceæ the chromophilous portions of the "central substance" contain iron, and iron may be also demonstrated in the peripheral granules containing the so-called cyanophycin. At this period, Macallum was specially concerned in proving the presence of organic iron in the chromatin of the nucleus and was not searching for iron in the chloroplasts, so that the reference above to the presence of iron in the cyanoplasts of the Cyanophyceæ is highly interesting to-day.

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* 'Roy. Soc. Proc.,' vol. 57, p. 261 (1894).

Molisch (*loc. cit.*) used long immersion in *saturated* potassium hydrate as a preliminary method for setting free masked iron (*i.e.* organic iron) in available form for after-detection by potassium ferrocyanide and hydrochloric acid, and in the later testing used very strong hydrochloric acid (10 to 20 per cent.). Such drastic procedures are very dangerous, because the alkali breaks down the delicate chloroplasts, and may also itself contain iron salts in traces; also in the second place, as pointed out by Quincke,* such strong acid will fairly rapidly set iron free in inorganic or ionic form from the potassium ferrocyanide reagent, and this ionic iron reacting with the remainder of the reagent will give the Prussian blue colour. Molisch found more iron in the epidermis and fibro-vascular bundles of green leaves than in the green mesophyll, but as he himself admits "the potassium hydrate so disorganises the nucleus and chlorophyll-granules that one can conclude nothing as to the distribution of iron in the cell."

So far as we have been able to discover there exist no records later than the above in the literature of the subject on the occurrence of iron in the chloroplasts of the green cell, nor any information as to the form in which iron compounds are present. No investigations with the iron hæmatoxylin test of Macallum appear to have been made hitherto upon plants.

Experimental Methods.

In carrying out tests for the detection of inorganic iron in the chloroplasts, and in plant tissues generally, two points must be carefully borne in mind, first, the previous preparation of the tissue and its subdivision so that the parts possibly containing iron may be penetrated by the reagents used for the detection, and secondly, that the reagents be applied carefully so that false results are not obtained. Here care must be taken with the concentration of the reagents and the relative periods of time within which positive results are obtained.

In regard to the preparation of the tissues, if sections are to be cut, care must be taken that this is done with a clean burnished knife. Control experiments show that a clean steel knife leaves no iron on the section. But, in most cases, since the question at issue is not the structural arrangement but rather whether this or that constituent contains iron, it is better to work with finely teased or broken up tissues. For this purpose glass rods drawn out to a point were always used instead of steel needles, and also, in order to break up some of the green cells and set free the chloroplasts, a portion of the tissue in each case was still more broken up by turning upon it the blunt end of the glass rod and grinding it between this and the microscope slide on which it was being mounted.

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* 'Arch. f. Exp. Path. u. Pharm.,' vol. 37, p. 183 (1896).

In choosing tissues for examination, preference so far as possible is given to those where the chloroplasts are more conspicuous in size, and also in some cases, such as *Spirogyra*, delicate filaments were chosen which could, after extraction as described below with alcohol, be mounted without breaking up.

In certain cases, such as *Pleurococcus*, staining can readily be obtained without previous chemical preparation of the tissue, but, in the majority of cases, the lipoids present along with the chlorophyll in the chloroplast prevent the penetration of the stain, also the green colour modifies and masks the blue of the hæmatoxylin in Macallum's test. For this reason it is well to remove the lipoids and chlorophyll, and in many cases this is by no means an easy task. In some cases standing in cold alcohol removes the chlorophyll quite effectually and leaves the tissue colourless and ready for staining; but in other cases the tissue may be left at ordinary temperatures for days in alcohol, and this may even be followed by several extractions with ether, and still some of the green colour remains. After a good deal of experimentation the best extractive in these latter cases was found to be boiling alcohol.

The tissue, either partially teased with the glass points if it is bulky like the leaf of a higher plant or a piece of lichen or moss, or left intact if a delicate structure like an algal filament or *Pleurococcus*, is placed in water in a watch-glass and then absolute alcohol is gradually added portion-wise and pouring away excess of the mixture at intervals until the fluid is finally all absolute alcohol. The preparation is then boiled in the alcohol and the greenish extract poured away, and this is repeated till the green tissue becomes colourless. The decolorised tissue is then brought back again into distilled water by gradually adding the water to the alcohol, and pouring off. Finally, it is allowed to stand a few minutes in a watch-glass in water redistilled from glass, and is then ready for staining.

In addition to the unmordaunted simple aqueous solution of well-washed hæmatoxylin in $\frac{1}{2}$-per-cent. concentration introduced by Macallum* the older histo-chemical tests for iron were also utilised, namely, potassium ferrocyanide and hydrochloric acid for ferric salts, potassium ferricyanide and hydrochloric acid for ferrous salts, and ammonium hydrogen sulphide in glycerine for both. In our opinion the Macallum test surpasses all these in reliability and delicacy. Its only fault is that it is too delicate, and the small traces of inorganic iron set free from organic compounds in the tissues on long standing cause faint but increasing staining when a preparation is left over for some days. When a blue-black is obtained, however, within a few hours with this reagent it is a decisive proof of loosely combined, or inorganic, iron in the situation where the staining occurs.

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* 'Journ. Physiol.,' vol. 22, p. 92 (1897).

Ammonium hydrogen sulphide when added to the tissues with an equal amount of glycerine, and the whole kept at 36° C. for some hours, produces a distinct blackening as compared with the normal, but the effect is not very pronounced and is only clear on comparison of treated and untreated tissue.

Potassium ferrocyanide and hydrochloric acid never gave a blue colour, but a blue colour is frequently, and very distinctly, given within a few hours by potassium ferricyanide and acid, demonstrating that the inorganic iron of the chloroplasts is present in the ferrous condition; this was typically observed in the case of *Spirogyra* and *Vaucheria.*

There is always some doubt, however, about using a reagent which itself contains the element sought for, and moreover is fairly readily broken down in presence of organic matter and acid.

The hydrochloric acid used should not exceed 0·5 per cent. in concentration, and be used in equal volume with the 1·5-per-cent. ferricyanide solution so that the concentration of acid acting on the tissue is only 0·25 per cent. Then, if a blue stain is obtained with a considerable intensity within 24 hours, it may fairly certainly be attributed to ferrous iron in that particular situation. The result, however, ought always to be confirmed by the Macallum test, for solid starch or casein left for 24 hours in contact with the above reagents each give a faint blue colour which increases as the mixture is left standing.

When a solution of hæmatoxylin in pure distilled water is mixed with a very dilute solution of an ordinary iron salt such as ferric chloride, a deep blue-black coloration is immediately produced. If, instead of an ordinary iron salt solution, a solution of highly colloidal or dialysed iron oxide be mixed with the solution of hæmatoxylin there is obtained instead a deep chocolate-brown coloration. In the course of some hours to a day or two, this chocolate brown is replaced by the blue-black colour obtained with ionic or crystalloidal iron salts. Similar results are usually obtained when the hæmatoxylin solution is used as a detector of iron in the tissue of plants. In certain cases, notably unicellular green plants and algal filaments, a deep blue-black is obtained within a few minutes without any previous appearance of the brown stain characteristic of colloidal iron oxide, while in many of the higher plants (mono- and dicotyledons) the green leaf at first stains a deep brown, which gradually, in a varying period of a few hours to a day or two, changes to a blue-black, just as is seen in the test-tube when colloidal iron oxide solution is mixed with the reagent. In certain cases, however, the brown colour is found to persist for weeks without change.

This deep brown coloration is not simply due to imbibition of the tissue with unaltered hæmatoxylin, for it is far too deep for this, and, moreover, is not removed by washing with a mixture of equal parts of alcohol and ether as recommended by Macallum. It is a true staining of colloidal iron, present in those parts of the tissues where the brown occurs, and possesses just the same dark brown colour that is obtained on mixing colloidal iron oxide solution and hæmatoxylin.

In contrast with vegetable tissues, such a direct staining (either brown or blue-black) is only found in the embryonic condition in the tissues of higher animals, for the iron in the majority of such animal tissues is firmly bound organically and gives no coloration with hæmatoxylin.

It is to be remarked that this staining as a test for iron is quite different from the ordinary use of hæmatoxylin as a nuclear stain in histological technique. In the ordinary use of hæmatoxylin as a staining reagent a mordaunt is always used either preceding the hæmatoxylin, as, for example, the iron alum mordaunt for Heidenhain's iron-hæmatoxylin method, or simultaneously as in the use of the hæmalum stain, where the mordaunt alum is mixed with the hæmatoxylin. But in Macallum's use of the stain no mordaunt whatever is used, but instead a simple aqueous solution in pure distilled water. This solution only strikes a colour where a mordaunt is naturally present in the tissue. Now with iron in colloidal form the colour struck is the deep brown mentioned above, with iron in crystalloidal form the colour struck is blue-black. Thus Macallum's method resembles Heidenhain's staining, but with the previous iron treatment naturally provided in the tissues, and the blue-black effect obtained closely resembles in many cases a Heidenhain iron-hæmatoxylin stain.

In order to use the method effectively, it is not merely necessary to avoid all minute traces of iron in the water and other fluids used, but also all traces of alkali and acid, since these interfere with the delicacy of the reaction. Alkali gives a rose-red colour with the hæmatoxylin, and acid inhibits the development of the blue-black when the amount of iron is small. In making up the stain itself, water twice distilled from glass vessels must be used as the solvent, the second distillation having been made immediately previous to use. To make the staining solution, 0·3 grm. of pure hæmatoxylin is weighed out, and washed with the twice distilled water till the crystals are colourless, and the wash-water is only pale yellow without any trace of red. The solution is then made up to 50 c.c. and kept in a Jena glass flask, for the alkali which is slowly dissolved out from ordinary glass rapidly turns the solution pink. The reagent should be pale yellow when used, in order to obtain the best effects, and does not keep in good condition for more than a few days.

After the chlorophyll and fats have been removed from the tissue by allowing it to stand in cold alcohol, or by boiling up with alcohol, the colourless tissue must be well washed with water, and the water used must, as described above, be doubly distilled from glass.

The staining process may be watched in progress, when it will be found that escaped chloroplasts from ruptured cells take on the stain first, and in many cases show a deep purple-blue within a few minutes. Within the intact cell the stain does not penetrate so rapidly, and the cell wall may show a blue staining in some cases before the contained chloroplasts, but eventually these also stain a deep blue, sometimes preceded by a dark brown. The nuclei of the green plant cells also stain a deep blue (unlike animal cell nuclei), and there is usually a much slighter diffuse blue in the remaining cytoplasm. The fibres associated with the vascular bundles also show in many cases a blue staining. This probably means that the iron salts are carried along this route to the green cells. But the early and deep staining of chloroplasts and nucleus are characteristic in the preparations.

In addition to tissues containing chloroplasts, several preparations have been made from plants not containing chlorophyll, such as yeast, moulds, and larger fungi. There is a marked contrast found here, a blue stain does not appear for some days, and then in only a comparatively feeble manner. The conidia and the conidiophores show more iron than the mycelium filaments. It is probable that this slow and feeble staining is due to organic iron compounds slowly breaking up and yielding traces of inorganic iron.

A series of ash analyses of chlorophyll-containing and chlorophyll-free plants show in all cases a much higher percentage of iron in the ash of the green plant; these analyses will furnish the subject of a separate paper.

A large number of plants of different types have been examined, and the main results are given in the following account.

Amongst unicellular green plants there were examined *Chlorella*, obtained as plankton from a green-coloured pond water; *Pleurococcus*, obtained in nearly pure condition growing on an oak fence near Oxford, and stained and examined in collaboration with Mr. Edward Whitley; several forms of diatom and several unicellular forms found in lichens.

The blue-black effect is very readily obtained with these unicellular green plants, often without previous removal of the chlorophyll. In the case of the lichens the contrast is marked between the green cells and the fresh hyphæ of the fungus, but dead or decaying fungal matter often gives a blue stain.

The algæ observed were species of *Vaucheria*, *Spirogyra*, *Ulva*, and *Ulothrix.* The effects were often repeated in several experiments, both with hæmatoxylin staining and with ferricyanide and hydrochloric acid. The ferricyanide solution does not appear to penetrate well, and only some filaments in an alga like *Spirogyra* are coloured, but the staining has been obtained within an hour or two of treatment with this reagent, and is a very beautiful effect when obtained in *Spirogyra.* The light blue colour follows the spirals of the chlorophyll bands, and the granules are obviously more deeply blue than the rest of the bands. The deep blue-black with hæmatoxylin is more readily and uniformly obtained, coming often within a few minutes of applying the stain to the decolorised alga, and furnishing again a beautiful effect. Sometimes, however, the brown colour of colloidal iron is obtained in *Spirogyra.*

Ulva latissima gives a very deep blue-black coloration, rendering the cells almost opaque ; its ash shows a high content in iron.

Cladothrix, when growing in water containing small amounts of iron, as is well known, secretes, or excretes, a tube of iron oxide around the filaments, and is then known as an "iron bacterium." When these so-called "iron bacteria" are treated with hæmatoxylin, they turn blue-black almost instantly, and, if the stained specimens are examined under the microscope, the interesting fact is immediately observable that not only the external tube, but the substance of the organism itself, is stained blue-black, so settling a much disputed point. The same is seen in *Vaucheria,* an incrustation of iron-oxide particles is demonstrable in the gelatinous sheath surrounding the filaments, either by ferricyanide or hæmatoxylin staining, but, in addition, both reagents show inorganic iron within the filament itself.

Many higher aquatic plants, such as *Lemna* and *Elodea,* possess such incrustations of iron oxide on their leaves when grown in water containing only traces of iron, but in such cases it is also found that the chloroplasts of the green cell itself are very rich in inorganic iron. The higher aquatic plants examined have been these two and a variety of water-cress, and all three were found to give a strong positive reaction.

Ordinary lawn grass contains a high percentage of iron in the ash, and, when teased out and deprived of chlorophyll by hot alcohol, forms a very suitable object on account of the ease with which strands of fibre with attached cells separate. The staining of the chloroplasts is at first a dark brown passing later into a blue-black. The leaves of many species of dicotyledonous plants were examined and it was found that here the transition from dark brown to blue-black was much slower as a rule, and in some cases the staining remained permanently of a deep orange-brown to a pure dark brown colour. But in all cases the chloroplasts stained more deeply than the remainder of the cytoplasm.

The catalyst for the photo-synthesis may not in all cases be an iron salt, or oxide, but an iron salt is present and capable of operating as a catalyst in a large number of instances.

Various substances known to be present in the ash of leaves have been tested for their photo-synthetic activity in connection with the work, and it has been found that magnesium and calcium phosphates and bicarbonates are entirely ineffectual, but that marked photo-synthesis of formaldehyde is obtained with chlorides or colloidal hydrates of iron or aluminium.

Summary.

1. Inorganic iron salts and iron or aluminium hydrates in colloidal solution possess the power of transforming the energy of the sunlight into chemical energy of organic compounds.
2. Inorganic iron, in crystalloidal or colloidal form, is present in the colourless part of the chloroplast of the green plant cell in many plants.
3. In the absence of iron the green colouring matter cannot develop in the leaf, although the green colouring matter itself contains no iron.
4. In the presence of sunshine, the iron-containing substance of the chloroplast develops the colouring matter, so that this itself is a product of photo-synthesis induced by the iron-containing compound.
5. These facts afford an explanation of chlorosis, and its cure by inorganic iron salts, and demonstrate that iron is a primary essential in photo-synthesis, and the production of chlorophyll.
6. The iron-containing substances of the colourless portion of the chloroplast, and the chlorophyll produced by them, then become associated in the functions of photo-synthesis as a complete mechanism for the energy transformation.

My thanks are due to my colleague, Prof. R. J. Harvey Gibson, for much valuable advice in selecting and obtaining suitable material, and to Misses E. M. Blackwell, M. Knight, and R. Robbins, of the Botanical Department of the University of Liverpool, for supplies of fresh material.

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CROONIAN LECTURE: *A New Conception of the Glomerular Function.*

By T. G. BRODIE, M.D., F.R.S., Professor of Physiology in the University of Toronto.

(Lecture delivered June 15, 1911,—MS. received December 9, 1912.)

[PLATE 26.]

I have chosen as the subject of this lecture the physiology of the kidney, and more particularly the mode of action of one part of it, namely the glomerulus. In 1906, at the meeting of the British Medical Association in Toronto, I brought forward a new conception of the action of this very characteristic portion of the renal apparatus, and since that time have been accumulating a considerable mass of evidence by the light of which my theory can be criticised.

Very shortly after the discovery of the main details of the structure of the kidney, Ludwig, basing his ideas upon the then known structure, put forward his well-known theory that the glomerulus was a filter, and since that time all discussions upon renal activity have centred round this theory because it offered an explanation of the mode of action of one part of the mechanism upon hydrodynamic principles. The necessary corollary following from this assumption of filtration is that a considerable degree of absorption must be effected as the dilute filtrate travels down the tubule, and how excessively great this must be was first pointed out by Heidenhain.

If we consider the results obtained by the earlier workers upon the kidney, very many of them appear sufficiently well explained by the Ludwig theory, but as in the course of years a far stricter examination of the theory was attempted, several observations were made which proved very difficult to explain, and in many cases it was necessary to make such extensive and often contradictory assumptions that it became increasingly difficult to accept the theory. Of recent years evidence has been obtained in many directions which in my opinion conclusively proves that the glomerulus is not a filtering surface. It is not my object to-day to discuss this point in any detail. I may refer to my lecture delivered before the Harvey Society in New York in December, 1909, where a short summary of the facts for and against filtration is given, or to the excellent paper by Magnus, in the 'Handbuch der Biochemie,' where it is discussed *in extenso.* It will be sufficient for my present purpose if I indicate the chief reasons which led me to conclude that the idea of filtration at the glomerular surface must be abandoned.

Perhaps the most striking piece of evidence is derived from the consideration of the concentration and constitution of the urines obtained during extremely free secretion. The evidence is quite clear that the main bulk of the water secreted by the kidney undoubtedly comes from the glomeruli. Hence the more rapid the flow of fluid from the kidney the more closely must that fluid resemble in constitution the fluid discharged from the glomeruli, since a much shorter time is then allowed to the cells of the tubules to modify it by absorption or secretion, and if filtration is the active process in the glomeruli this fluid ought to approximate more and more closely in composition to the blood plasma so far as the salts, urea and all constituents of the plasma other than proteins are concerned. But the dilute urine secreted after drinking copious amounts of lager beer,* or of water,† shows a constitution in salts widely different from that of the blood. Considering only the total concentration, as estimated by the depression of the freezing point, it is quite easy to obtain a urine with $\Delta = -0{\cdot}1^\circ$ C., and one as low as $-0{\cdot}075^\circ$ C. has been recorded.‡ To effect a change in concentration so extensive as this denotes, by filtration through a semipermeable membrane, would necessitate a pressure difference on the two sides of the membrane of at least 4000 mm. Hg, a pressure difference utterly out of comparison with the blood-pressure. Therefore to make such a result accord with the filtration theory, it becomes necessary to assume a most extensive reabsorption of the salts and other substances of small molecular size, a reabsorption on such an extensive scale and at such a rate as is, I think, entirely out of the question.

If, in the second place, we investigate the correlation between the blood flow and the rate of secretion, we find that while there is a general correspondence, in that increased urine flow is usually accompanied by increased blood flow, this is by no means a universal rule.§ I have frequently observed in kidneys in which there was at the start a fairly free blood flow and but slow urine secretion, a copious diuresis to come on without any change in the blood flow. Indeed on no less than five occasions I have seen a distinct decrease in the blood flow to occur as the diuresis commenced, and moreover in these experiments the volume of the kidney actually increased. In every direction we find that the urine flow does not vary strictly with the blood flow nor with the blood-pressure, as should be the case were filtration the essential factor in determining the volume of the urine discharged from the kidney.

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* Dreser, 'Arch. Exp. Path.,' 1892, vol. 29, p. 303.

† Macallum and Benson, 'Journ. Biol. Chem.,' 1909, vol. 6, p. 87.

‡ Macallum and Benson, *loc. cit.*

§ *Cf.* Gottlieb and Magnus, 'Arch. Exp. Path.,' 1901, vol. 45, p. 223.

In the third place we have very decisive evidence against the Ludwig theory in experiments designed to test the second assumption in that theory, namely that of reabsorption. If this is a process which occurs extensively within the tubules, and we bring into play any factor which favours reabsorption, we ought to effect a diminution in the volume of urine yielded by the kidney. Such a factor is an increase in hydrostatic pressure within the ureter, tending to prevent the outflow of urine. All that is necessary is to make the kidney discharge against a small pressure. The experiments carried out by most experimenters upon these lines have indeed yielded results which may be interpreted as indicating increased reabsorption. But we may urge as a general criticism against such results that the degree of decrease of urine flow is surprisingly small when we remember how essential it is according to Ludwig's theory to assume that reabsorption is excessively free. The kidney working against even a small hydrostatic pressure ought to show far greater reabsorption than was actually obtained. But the whole idea of reabsorption as an active process in the formation of urine has been completely disproved by Miss Cullis and myself,* for we were able to prove that decrease in rate of the urine flow when a kidney was made to secrete against a pressure was only a universal result when the animal was under an anæsthetic, and that if the animal were pithed and the experiment then performed in the absence of an anæsthetic, the kidney working against a small pressure always excreted more salt and usually more water than the opposite kidney.† The action of a pressure then tends to excite the kidney to greater activity, a result which entirely disproves the possibility of reabsorption being an extensive factor in the normal formation of urine.

Yet another point which militates greatly against the idea that the glomerulus is a filter is the behaviour of the kidney after temporary asphyxiation. If the renal artery be clamped for one minute and then released, the kidney does not at once begin to secrete, although the blood flow returns at once. It is only after a variable, but usually considerable delay that the kidney restarts, and at first the urine flow is very slow, only gradually returning to a rate comparable to the initial flow. If the artery has been clamped for any length of time the urine first collected after the re-establishment of the circulation contains protein, casts, even hæmoglobin, indicating considerable damage to the renal epithelium, either of the tubules or of the glomeruli or of both. But even if the glomerular epithelium be damaged it is inconceivable that this should temporarily abolish all the filtering properties it formerly possessed, and it is just as difficult to understand why the recovery of its power to filter should occur so gradually when the asphyxiation is arrested.

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* Brodie and Cullis, 'Journ. of Physiol.,' 1906, vol. 34, p. 224.

† Subsequent to these experiments I have found that, under the same conditions, the blood flow through the kidney is not altered by the small rise in ureter pressure employed in our experiments.

Let us next turn to the evidence that has been sought in favour of Ludwig's theory from experiments upon the maximum ureter pressure. One of the earliest attempts to associate the formation of urine directly with the blood-pressure was a measurement of the maximum height to which the kidney could force the urine up a vertical tube. As is well known, in the case of the salivary gland, the gland can secrete water to a pressure exceeding that of the blood in the carotid artery, a clear indication that a new force, viz., one exerted by the salivary gland cells, is at play in producing the result. But in the case of the kidney the result is very different. For the maximum ureter pressure always lies below the aortic blood-pressure, and usually some 30–40 mm. Hg below that pressure. The results were therefore interpreted by supporters of the filtration theory as indicating that as soon as the pressure within Bowman's capsule reached a point some 30 mm. Hg below the glomerular blood-pressure, filtration ceased, and Starling* explained the difference between the aortic pressure and the maximum ureter pressure as being the pressure difference necessary for the separation of the blood proteins from plasma, for he estimated the osmotic pressure of the blood protein at that amount. It has since been shown, however, that the protein osmotic pressure is certainly much less than this. Moreover Starling failed to allow for a loss of pressure between the aorta and the glomerular capillaries. Without doubt the loss of pressure between these points is less than in the case of ordinary capillaries, for the resistance in the kidney arterioles when dilated is certainly much less than at most points on the systemic circulation. As I shall show later, the maximum ureter pressure as ordinarily taken is a measure of the blood-pressure in the glomerular capillaries.

But a still more difficult problem is offered to those accepting the filtration theory in explaining these experiments. As was first pointed out by Heidenhain,† upon the Ludwig theory the maximum ureter pressure should be that pressure which just suffices to effect complete reabsorption of all the glomerular filtrate. Upon the theory we are to imagine an absorbing surface, capable of absorbing water, chlorides, urea and most of the bodies filtered in urine at a very fast rate. Such an absorbing surface would be influenced, as indeed is usually assumed by the supporters of Ludwig's theory, by a rise in pressure of the fluid at the surface. It then becomes very difficult to explain how the ureter pressure could ever be driven so high as is usually observed, especially when we remember that the rise in pressure can be effected with great rapidity.

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* Starling, 'Journ. of Physiol.,' 1899, vol. 24, p. 317.

† Heidenhain, 'Hermann's Hdb.,' vol. 5, p. 327.

Yet another result obtained in these experiments upon maximum ureter pressure is very significant. I have found that the maximum ureter pressure is practically the same whether the kidney be made to secrete a moderate amount of urine or a very large quantity. If reabsorption be a very active process, then the maximum ureter pressure in the latter case ought to be distinctly higher than in the former. As a matter of fact, it is not.

Taking everything into account, therefore, I have very grave doubts as to the occurrence of reabsorption in the tubules, and I am sure, if it does take place, that it is insignificant in comparison to that demanded by Ludwig's theory.

The Function of the Glomerulus.

Arriving then at the conclusion that the filtration theory was incorrect, I came back once more to the old problem: How are we to explain the very peculiar and characteristic structure shown by the glomerulus? I finally hit upon the idea that it was simply a means of utilising the blood-pressure for setting up a pressure head sufficiently great to drive the urine secreted at the glomerular surface down the tubule. To express this idea I term the glomerulus a propulsor. As is abundantly proved, the main volume of the water of the urine is secreted into the capsule of the glomerulus. To drive it from the capsule down the tubule requires a definite pressure-head. Whence is this pressure head derived? My view is that the intraglomerular blood-pressure is transmitted directly through the thin-walled glomerular loops to the fluid which has been secreted into the capsule, and thus a pressure is communicated to the fluid sufficient to force it down the tubule. To test this view, let us imagine that a certain amount of fluid has accumulated within Bowman's capsule. The problem then becomes: How is that fluid discharged down the tubule? If we know the number, length and lumina of the tubules, and the total amount of fluid leaving the kidney within a given time, it becomes easy to calculate the pressure-head which must have existed within each capsule in order to drive the fluid out of the kidney. It is simply an application of Poisseuille's law. I therefore performed two experiments upon the following lines. An active diuresis was established in an anæsthetised dog, and the rate at which urine was being discharged from one of the kidneys was determined. The pedicle of the kidney was then ligatured and the kidney fixed entire in 10-per-cent. formalin solution. After fixation the whole kidney was cut into slices each about 7 mm. thick. The medulla was carefully separated from the cortex, and the latter collected and weighed. Next three small pieces of the cortex, selected from different regions of the kidney, were weighed separately. These were imbedded in paraffin and serial sections mounted. The sections were about 8μ thick. The next point was to determine the number of sections through which a single glomerulus extended. For this purpose ten glomeruli were followed through the series, and the mean number of sections through which one glomerulus ran thus ascertained. Lastly the total number of glomeruli in each section was counted, and the total number for all sections, divided by the average number for a single glomerulus, gave the total number of glomeruli present in that block of cortex. Similar calculations were made from each of the other two pieces. Then, knowing the weights of the three pieces and the total weight of the cortex, the number of glomeruli in the whole kidney was obtained.* The first dog weighed 11 kgrm., its right kidney weighed 34·5 grm., and the total number of glomeruli was 142,000. A kidney of a second dog, weighing a little over 8 krgm., contained 125,000 glomeruli.

Employing a different method, Peter† calculated the number of glomeruli in the dog's kidney as 300,000. He does not give the weight of the kidney, nor does the method he employed appear to me comparable in accuracy with that above described. I have not been able to find any further record of enumerations of the glomeruli in the dog's kidney, and I wish to acknowledge my great indebtedness to Miss M. G. Thackrah for carrying out this very tedious piece of work.

Measurements of the lumina of the tubules in their several parts were now made, as also approximate estimates of the lengths of the tubules based upon the measurements of Peter.

The average results obtained from these measurements in the case of the first kidney were :—

	Length. cm.	Diameter. μ.
Proximal convoluted tubule	1·2	12
Loop of Henle—		
Descending limb	0·9	10
Ascending limb	0·9	9
Distal convoluted tubule	0·2	18
Collecting tubule	2·2	16

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* This is practically the method originally adopted by Huschke in 1828 ('Isis,' vol. 21, p. 550).

† Peter, 'Verhandl. D. Anat. Ges., Würzburg,' 1907, p. 120.

The diuresis at the time the kidney pedicle was ligatured was 1 c.c. per minute.

From the formula for the flow of liquids along narrow tubes

$p = 8l\eta/\pi r^4$ times flow in cubic centimetres per second dynes per square centimetre,

where l is the length of the tubule in centimetres,

η is the coefficient of viscosity, and

r is the radius of the tube in centimetres.

Taking η as 719×10^{-5}, the coefficient of viscosity of water at 35° C., we have

$$p = \frac{8 \times 719 \times 10^{-5}}{\pi} \cdot \frac{1}{60} \cdot \frac{1}{142000} \cdot 10^{16} \cdot \frac{l}{r^4} \text{ dynes per square centimetre,}$$

r being now expressed in microns; or

$$p = \frac{8 \times 719 \times 7}{22 \times 6 \times 142 \times 1333{\cdot}2} \cdot 10^{7} \cdot \frac{l}{r^4} \text{ mm. Hg.} = 1{\cdot}611 \times 10^{4} \times \frac{l}{r^4} \text{ mm. Hg.}$$

Consequently, for a flow of 1 c.c. per minute,

	μ.	mm. Hg.
p per centimetre of tubule, when	$r = 4{\cdot}5,$	$= 39{\cdot}29,$
	$r = 5,$	$= 25{\cdot}78,$
	$r = 6,$	$= 12{\cdot}43,$
	$r = 8,$	$= 3{\cdot}93,$
	$r = 9,$	$= 2{\cdot}46.$

Hence pressure-head required for—

		mm. Hg.
Proximal convoluted tubule......	$= 1{\cdot}2 \times 12{\cdot}43 =$	14·916
Loop of Henle—		
Descending limb	$= 0{\cdot}9 \times 25{\cdot}78 =$	23·212
Ascending limb..................	$= 0{\cdot}9 \times 39{\cdot}29 =$	35·361
Distal convoluted tubule	$= 0{\cdot}2 \times 2{\cdot}46 =$	0·492
Collecting tubule	$= 2{\cdot}2 \times 3{\cdot}93 =$	8·646
Total pressure-head.........		82·627

In the case of the second kidney, with 125,000 tubules, the measurements were:—

	Length. cm.	Diameter. μ.
Proximal convoluted tubule......	1·0	12
Loop of Henle—		
Descending limb	0·8	10
Ascending limb	0·8	10
Distal convoluted tubule	0·2	18
Collecting tubule	2·0	8

And, with a diuresis of 0·85 c.c. per minute, the pressure-head required works out to 74·1 mm. Hg.

I do not wish to lay too great a stress upon the actual pressure-head thus obtained, for the possible errors in the measurements are many. It is, for instance, impossible to obtain anything but an approximation to the lengths of the successive portions of the tubule, and also the measurements of their lumina can only be approximate, for they are undoubtedly altered during fixation. Also I have supposed all the tubules to have equal lumina, and have neglected to take into account those tubules which were at rest. To obtain the total pressure within Bowman's capsule a factor for the velocity head should be added to the pressure-head already calculated, but it is so small that we may omit it. (The mean velocity within the narrowest portion of the tubule amounts to about 1 mm. per second.)

The important point is that during an active diuresis a pressure-head of the order of 80 mm Hg. may be needed within Bowman's capsule to drive the fluid secreted there down the tubule.

The mean aortic blood-pressure in the first experiment was 120 mm. Hg, and in the second 115 mm. If we allow 30–35 mm. Hg as the loss of pressure-head between the aorta and the glomerular capillaries when the afferent glomerular vessels are dilated, the blood-pressure within the capillary loops would amount to 90–85 mm. Hg in the first experiment, and 85–80 mm. in the second. Hence, on these figures, practically the whole of the blood pressure-head is required to set up a pressure-head in the fluid within the capsule sufficient to drive the secreted fluid down the tubule. Bearing in mind that the estimates given are only approximate, I conclude that the pressure-head within Bowman's capsule only differs from the pressure-head within the glomerular loops by the pressure required to stretch the walls of the loops. This latter probably does not amount to more than one or two millimetres of mercury.

If in the light of these arguments we criticise once more the assumptions made by Ludwig's theory, we see that that theory becomes less tenable than ever. In the first place, when the kidney is secreting water at its fastest rate, the pressure difference available for filtration is reduced to a minimum At lower rates of secretion, of course, a pressure difference might be available. In the second place, the assumption must be made that the volume of water discharged from the glomerulus is from 30 to 70 times greater than the volume of water entering the pelvis of the kidney. Hence a very much greater pressure-head would be required to drive that fluid down the tubule, though not 30 to 70 times greater than the pressure required to drive a volume equal to that of the discharged urine, since the fluid has to be driven only as far as the absorbing surface. But as the absorbing surface would have to be taken as extending at least to the end of the ascending limb of the loop of Henle, *i.e.* along considerably more than one-half of the whole tubule, and the whole length of the narrowest part of the tubule, the pressure-head required would be enormous, certainly many times greater than the glomerular blood-pressure. We should, therefore, be compelled to ascribe to the cells secreting the water the power of setting up a very high hydrostatic pressure, and all the evidence is strongly against any such view. A pressure within Bowman's capsule greater than the blood-pressure would at once lead to the closure of the glomerular loops and arrest of the circulation. This is the main reason why neither the cells of Bowman's capsule, nor these covering the glomerular tufts, nor those of the convoluted tubule, possess the power of setting up a hydrostatic pressure.

The quantity of energy imparted by the blood to the glomerular secretion is only a small percentage of its total amount. Thus if V be the minute volume of blood flowing through the glomerulus, and v the minute volume of glomerular secretion, then V c.c. of blood enter the glomerular capsule, and $V-v$ c.c. leave it. If p be the pressure-head in the glomerular loops, the pressure energy of the blood entering is Vp, and that of the blood leaving is $(V-v)p$. The pressure energy communicated to the glomerular secretion is vp, and the ratio of this to the total pressure energy of the blood as it enters is v/V. In the dog's kidney V may have any value from 200 to 600 c.c., and v from 1 to 2 c.c. at the height of a diuresis. Thus the pressure energy given up by the blood lies somewhere between 1 and 0·16 per cent. of its total pressure energy.

Histological Evidence.

In the next instance the test applied was that of microscopical examination of the kidney after varying degrees of activity. If during diuresis fluid is being forced at a considerable pressure from Bowman's capsule down the tubule, evidences of the action of this pressure should be indicated by changes both in the glomerulus and in the tubule. It is very remarkable that throughout the literature the accounts of changes in the glomerulus following activity are so scanty, and many authors state that no changes whatever are to be found (*e.g.* Lamy and Mayer). Mackenzie and I therefore examined a number of kidneys excised after diuresis had been induced under various conditions, and found that decided changes are produced in the glomerulus and tubule. We further found abundant evidence proving that the tubules have been subjected to a high internal pressure. The full details of these changes are given in a separate paper.* The general results are as follows:—

On comparing a resting kidney with one that has been thrown into activity by the injection of any diuretic which causes a free flow of water, the differences between both glomeruli and convoluted tubules are of the most striking character. These differences are illustrated in figs. 1 and 2, which show the changes in the cortex under a low magnification. The important points are the following:—In an active kidney the glomeruli are always separated from the capsules, and usually there is a considerable accumulation of fluid in this position. The capsule is always rounded, whereas in the resting kidney the capsule lies in contact with the glomerulus, and the whole structure is usually irregularly polyhedral in shape. In an active kidney, in contradistinction to the resting, the individual loops of the glomerulus are frequently separated from one another and stand out clearly. The glomerulus also has a very characteristic vacuolated appearance, due, we think, to dilated capillaries, from which the red blood corpuscles have in some way or other been removed or destroyed, possibly *post mortem*. When examining two such kidneys under a low power of magnification the contrast is most striking. In the resting kidney the glomeruli are far from conspicuous, and have to be sought for. In the active kidney, on the other hand, they stand out at once as the most conspicuous objects in the field of view.

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* *Vide* p. 593.

The changes in the tubules are just as striking. Whereas a resting proximal convoluted tubule possesses no lumen, one in activity has a large lumen. This is true both of the proximal and the distal tubules. Moreover, in the resting kidney the tubules are very much twisted on themselves and form very complicated foldings, whilst in the active kidney the appearances indicate that the tubule is as far as possible straightened out. All these several points prove quite clearly that the tubules have been subjected to some high fluid pressure from within.

The changes accompanying activity are strikingly emphasised when we measure the diameters of these several structures. In the case of the glomeruli and capsules, in addition to measurements in diameters at right angles to one another, approximate calculations of their volumes were also made.

In one experiment which we may take as typical we obtained the following results:—

	Resting.	After activity.
Volume of capsule............	83*	220
„ glomerulus	80	111
„ fluid in capsule	3	109

* These figures can be converted into cubic millimetres by multiplying them by $4{\cdot}2 \times 10^{-6}$.

The differences are therefore very great. The capacity of Bowman's capsule in the active kidney is nearly three times that of the capsule in the resting kidney, chiefly on account of the big accumulation of fluid within the capsule.

The volume of the glomerulus has also increased, though only by 40 per cent. Such measurements prove, therefore, that both the glomerulus and the capsule of Bowman are extensible structures, and that a considerable volume of fluid accumulates in the capsule during activity.

In drawing deductions from these measurements, full attention must be paid to possible alterations occurring after the kidney is excised. To obviate change as far as possible in these experiments, the artery, vein and ureter were ligatured close to the hilum at the instant the experiment was to be stopped, using a single coarse ligature. The kidney was then excised, rapidly weighed, and placed at once in the formalin fixative. If active diuresis were in progress, the kidney at the moment of ligature was hard and tense, but within a few seconds after application of the ligature became quite soft, chiefly on account of escape of blood through the Capsule. We found it impossible to avoid this. The question therefore arises: Does this fall of tension within the kidney substance involve a change in distribution of the fluid contained within the tubule and capsule? It is possible, for instance, that fluid is forced back from the distended tubule into the capsule. Possibly this may be the cause of some of the increase in volume of the capsule seen in our experiments, but the changes are too great to be wholly, or even largely, explicable in this way. There is yet another *post-mortem* change we think possible, viz., that before the fixative has time to penetrate and reach the glomeruli, the cells forming the loops die and permit osmotic effects to take place through them between the fluid in the capsule and the blood. Fluid would pass into the blood, and we think it possible that this fluid is so low in salinity as to lake some of the corpuscles, thus producing the vacuolated appearance described above.

In the same experiment the measurements of the diameters of the proximal and distal convoluted tubules and of their lumina were as follows:—

	Resting. μ.	Active. μ.
Proximal convoluted tubule—		
Transverse diameter	44·0	43·0
Lumen, diameter	0·0	19·4
Distal convoluted tubule—		
Transverse diameter	25·4	31·8
Lumen, diameter	11·0	21·8

This is fairly typical of the results obtained in all our experiments. We found it to be practically a universal rule that the external diameter of the proximal convoluted tubule remained unaltered, or showed but a slight increase or decrease. The marked change during activity is the production of a big lumen within the proximal tubule. The idea given by an examination of the sections is that the loops of the convoluted tubule have been opened out and stretched in length. They are in nearly all instances circular in outline, and invariably, as just stated, there is a very wide lumen. The distal convoluted tubule in contradistinction is nearly always increased in diameter in the active state, and the lumen greatly increased, often doubled, although this tubule has invariably a rather large lumen even in the resting kidney.

We have not yet carried out a sufficient number of measurements of the remaining portions of the tubule to warrant us making any decided statement as to the changes they undergo. It is clear that the limbs of the loop of Henle are both distended, and often the collecting tubules show very distinct expansion.

The next modification in our experiments consisted in comparing the two kidneys after active diuresis, one kidney having been previously stripped of its Capsule.

The kidney is very characteristically enclosed in a strong and practically inextensible Capsule*, and my view of the meaning of the glomerulus offers an explanation of that fact. As fluid is secreted into Bowman's capsule by the epithelium covering the glomerular loops, and possibly also by the epithelium of the capsule, the blood-pressure acting within the glomerular loops is transmitted directly to that fluid and through it to the wall of Bowman's capsule. This latter, as we have seen, is extensible and might be ruptured if the distension were carried too far. Again, fluid is at once forced into the convoluted tubule, and that also might be ruptured if overdistended. To prevent any dangerous overdistension the whole of the structures are enclosed in a firm Capsule. That this distension does take place on activity is amply proved in a variety of ways. Firstly, as shown above, the histological appearances demonstrate it. Secondly, if in an experiment we excise one kidney at the commencement, then excite diuresis, and at its height ligature the pedicle of the other kidney to prevent escape of urine from the tubules, and we then weigh the two kidneys, the latter often shows an increase in weight amounting to about 30 per cent. This increase in weight is not due to blood, for on excision the blood escapes more readily from such a kidney than from a resting kidney. In the third place I have often observed the following changes during the course of an oncometric experiment, viz., a large increase in the volume of the kidney, a free flow of urine, but a decrease in the rate of blood flow through the kidney. Here the plethysmographic increase is due to an accumulation of urine within the capsules and tubules. Lastly, if we examine a kidney at the height of a diuresis we always find it very hard and tense. The Capsule is distended to its fullest degree. If we attempt to make such a kidney expand still further by temporarily clamping the vein we fail completely. We see then that some of the tension set up by the blood-pressure in the glomeruli is transmitted through the capsule wall and the walls of the tubules to the general renal tissues. How much pressure is thus transmitted depends upon the resistance to distension offered by Bowman's capsule and the walls of the convoluted tubules. Their structure, particularly that of the capsule, indicates that they probably offer a fairly considerable resistance. We could get an estimate of this by finding the difference between the blood-pressure in the glomeruli and the general tension of the kidney substance within its Capsule. I made some attempts to measure this latter during active diuresis, but at present have not obtained any very accurate results. As far as they go they indicate a tension of about 40 mm. Hg.

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* In order to avoid confusion between the Capsule of the kidney and Bowman's capsule, I will when referring to the former distinguish it by a capital.

If this be the true meaning of the kidney Capsule then, if we remove it before exciting diuresis, the kidney ought to expand still further as compared to the intact one, and the amount of that further expansion should depend upon the general rigidity of the kidney substance and the amount of connective tissue it contains. Our experiments proved this to be the case. The weight of such a kidney compared to one with the Capsule untouched was always greater, especially in the rabbit's kidney. In the cat there are a number of incomplete septa running transversely towards the hilum, and on active diuresis the kidney substance bulges notably between these, giving the appearance of constricted grooves in the bottom of which veins run. This relatively greater increase in volume of the kidney as a whole is also found in the several parts of the tubule, and when we measured the tubules and glomeruli in such kidneys, the differences were very distinct. For instance, in one experiment the right kidney was untouched, and the left decapsulated. The following approximate volumes of the capsule and glomerulus after diuresis were obtained:—

	R.	L.
Volume of capsule	205	257
„ glomerus	128	151
„ fluid	27	106

The diameter of the tubules was as follows:—

	μ.	μ.
Proximal convoluted tubule—		
External diameter	44·8	48·0
Lumen	13·0	19·8
Distal convoluted tubule—		
External diameter	33·2	39·2
Lumen	24·8	29·2

The expansion then is found in all parts, and is obviously brought about by a distending force acting within the tubules.

Yet another means of testing the theory which presented itself was to observe the effect of obstructing the exit of urine down the ureter. In the first set of experiments a diuresis was set up, and at its height the ureter on one side was suddenly clamped. Five to fifteen minutes later the two kidneys were exposed, their condition noted, and then the pedicles ligatured as close as possible to the hilum. The kidneys were then removed and weighed. As was to be expected, a kidney obstructed in this manner is very distended and tense within its Capsule. The weights found in one experiment in which the right kidney was obstructed at the height of diuresis, and the left secreting freely, were as follows:—

	grm.
Weight of R. kidney	15·5
„ L. kidney	13·5

The right kidney was very tense, appeared almost bloodless, and was distinctly lobulated. The left kidney was distinctly softer than the right and also more vascular. The approximate volumes of the capsules and glomeruli were:—

	L.	R.
Volume of capsule	86	146
„ glomerulus	69	89
„ fluid	17	57

The measurements of the tubules were:—

	μ.	μ.
Proximal convoluted tubule—		
External diameter	39·2	39·4
Lumen	6·6	14·0
Distal convoluted tubule—		
External diameter	22·8	28·2
Lumen	12·6	18·4

Lastly, in an experiment in which an obstructed kidney was compared to a decapsulated one we found that the former procedure produced more effect than decapsulation.

Maximum Ureter Pressure.—Another series of observations which receive a satisfactory explanation is that in which the maximum ureter pressure is measured. According to my theory, fluid should be forced out of the tubules only when the pressure within the ureter lies below the maximum glomerular blood-pressure. This of course assumes that the tubular epithelium in secreting does not set up any appreciable hydrostatic pressure. From this point of view the measurement of the maximum ureter pressure should be a means of determining the intraglomerular blood-pressure, always supposing that none of that pressure is taken up by the walls of the glomerular loops. Now the measurements of the maximum ureter pressure fit in perfectly with this conception. In an animal whose aortic blood-pressure is about 120 mm. Hg, the maximum ureter pressure is usually found to be somewhere between 80 mm. and 90 mm. Hg, that is, a loss of pressure-head of some 30 to 40 mm. Hg occurs between the aorta and the glomerular capillaries. This is distinctly less than is the case for most systemic vessels, and fits in well with our knowledge of the relatively wide and short path of the blood stream from the aorta to the glomerulus. We have only to recall how fast the blood may flow through the kidney to realise that the glomerular capillary pressure during activity must stand at a greater height than the ordinary systemic capillary pressure.

Let us then return to a general restatement of the whole problem. I have given evidence that the glomerulus, Bowman's capsule and certain parts of the tubules are elastic structures, and that their overdistension is prevented by the general inextensibility of the connective tissue framework and of the Capsule. Consequently as soon as fluid is secreted by the glomerular surface into the capsule, the glomerular capillary pressure comes into play, and some part of that pressure is transmitted through Bowman's capsule to the tubules immediately outside. Then as the secretion continues to accumulate, the kidney expands to fill the Capsule, and the pressure within the Capsule reaches its maximum. Hence we may regard the glomeruli as a number of expanding vascular tufts, lying within a space which cannot expand beyond a certain point, consequently the expansion of the glomeruli expels any fluid free to move outwards. It is as if we were dealing with a sponge work filled with fluid, and enclosed in a capsule which it completely fills. Distributed through the sponge are a number of elastic structures which can be expanded by a fluid pressure acting from within, their expansion necessarily compressing the sponge, *i.e.* expelling the fluid from between its interstices. This analogy is of course incomplete, in that it takes no account of the tubular structure and the facts that the pressure is set up in the fluid within the tubules and that the walls of the tubules offer some resistance to expansion. The first effects of the glomerular pressure will therefore be to distend the capsule and the first convoluted tubule, *i.e.* to increase its lumen, thus offering less resistance to the flow of fluid along the tubule. In this distension the pulsation of the glomerular vessels is probably utilised. Also the more rapid the flow along the tubule the greater the pressure gradient, and the smaller the pressure transmitted through the walls of the tubules to the general kidney substance. We must therefore expect to find a distinct difference between the intratubular pressure and the intra-Capsular pressure, and while fluid is moving down the tubule the two could only be equal at the point where the tubule leaves that part of the kidney substance where the pressure is raised. This region is limited as we shall see by the branching arches of the renal vessels in the intermediate zone.

There is yet another feature of the renal structure and form which is capable of interpretation by this theory. This is the general shape of the mammalian kidney, so typical as to give its name to all structures in any way resembling it. The kidney is very typically constructed of a cortical mass enveloping a medullary portion. The blood-vessels form a set of arches between these two parts. My suggestion is that this arched system of vessels forms a more or less rigid base upon which the cortex lies. Consequently when, in activity, the pressure in the general renal tissue rises through the activity of the glomeruli it is restricted in the first instance to the cortex. The cortex, so to speak, becomes compressed between the rigid Capsule and the firmly distended arterial arches. From this general pressure the medullary portion is relieved, and it is a most significant fact that the loops of Henle lie within this region, where there is probably but little external pressure. Apparently, then, the difference in state between the tubules in the cortex and those in the medulla is that there is a high pressure on both internal and external surfaces of the tubules lying in the cortex, whereas in the medulla the pressure may be acting chiefly, possibly entirely, from the inner surface of the loops only. In this connection I have frequently observed the following most notable result:—If at the height of a diuresis whilst urine is flowing freely the ureter be ligatured, and after about 20 minutes the pedicle be tied off and the kidney removed, it will be found that the pelvis is widely distended with fluid, and usually the pyramid is compressed towards the cortex until it forms an almost insignificant structure projecting into the cavity of the pelvis. Histologically the tubules within such a collapsed pyramid are observed to be flattened and empty.

It is possible that some or even all of this compression might be *post mortem*, but I think that it is *ante mortem*, since it is only found if sufficient time be allowed to lapse between the ligaturing of the ureter and the removal of the kidney. The longer the interval the more marked is the compression. I think the compression is produced in the following way:—After the ureter has been ligatured urine continues for a time to be expelled into the pelvis, and gradually the pressure there rises. Fluid will continue to be forced into the pelvis in gradually decreasing volume until the pressure reaches that of the glomerular capillary blood-pressure. The further distension of the pelvis and compression of the medulla is probably produced through the pulsatory variations of pressure in the cortex. The systolic pressure, by the expansion of the glomeruli and arteries, suddenly raises the tension throughout the whole cortex; this expels a little of the fluid from the terminal portions of the tubules into the pelvis, whose pressure then becomes greater than diastolic pressure. As the pressure falls in diastole a point is reached at which the cortical pressure is below the pressure in the pelvis, that is below the pressure in the fluid contained within the loops of Henle and the collecting tubules. Accordingly these latter are emptied or partially emptied into the cortical tubules, while the lower ends of the collecting tubules are compressed and act as valves, preventing any return flow from the pelvis up the tubule. In this way more and more fluid is gradually collected within the pelvis at the expense of the medulla.

If, as I think is the case, we may divide the kidney substance into two parts, in one of which the whole tubule is exposed to a considerable pressure, both internal and external, while in the other region the pressure is largely within the tubule, the difference must have some important physiological meaning. It is most significant that the loops of Henle are carried down into this region of low external pressure. In different animals the loops of Henle show many diversities of form, more particularly in length, and it is certainly a striking fact that in some animals the major number of loops are short, and either lie completely within the cortex or only descend into the outermost portions of the medulla. It has been pointed out that the animals with very short loops are those which secrete a dilute urine, whilst those in which the loop penetrates far into the medulla secrete a concentrated urine. Hence it may be that this loop effects a certain amount of absorption, a function which would be aided by a pressure difference acting from within the tubule.

To test my theory further, and in the hope of gaining some evidence of the respective activities of the different parts of the renal apparatus, another series of experiments was performed, in which the action of diuretics upon animals whose blood-pressure had been lowered by section of the spinal cord was tested. It was necessary to employ rabbits for these observations, since in both the cat and the dog the blood-pressure remains high enough after section of the cord to enable the kidney to secrete quite freely when a diuretic is administered. In the rabbit the blood-pressure falls to about 30 mm. Hg, and even though we injected large doses of saline and other diuretics we never obtained a single drop of urine from the kidneys. The plan of experiment therefore was to excise one kidney some 10 to 20 minutes after division of the spinal cord, then inject the diuretic to be studied, and half-an-hour later to remove the other kidney. In this way evidence was obtained indicating the point of action of various diuretics. Without going into the results in detail, I may state that the glomerulus is excited to secrete by most of the diuretics of the saline group. Thus activity was well marked after sodium sulphate, urea, or dextrose; it was excited also by caffeine, but completely absent after phloridzin. In the tubules the results were equally striking, especially in the case of phloridzin, and in a minor degree in the case of caffeine. In no instance was a large lumen produced, and the external diameters of the convoluted tubules were only slightly increased. The contents of the lumen consisted of fairly large secretion droplets, the droplets being enclosed in membranes which stained with Weigert's hæmatoxylin, and fairly well with eosin. These results were chiefly observed in the proximal convoluted tubule. With the low blood-pressure there was never the slightest indication of any marked distension of the tubule in any part of its course. The glomeruli were never found secreting very actively, but were always found separated from the capsular epithelium by a distinct though small accumulation of fluid.

An examination of the embryology of the renal tubule bears out the views I have expressed. Originally, the excreting apparatus was a long tubule opening at one end into the body cavity, and at the other on to the surface. This tubule was lined throughout by a ciliated epithelium, which provided the necessary motor mechanism for the expulsion of the secretion. Later, the glomerulus was developed from the dorsal wall of the body cavity and received a large and important blood supply from the aorta. Possibly its original function was to secrete a watery fluid into the body cavity, and this in some way served the renal tubule. The arrangement of its vessels as large loops projecting from the cœlomic wall, even at this early stage, tends to indicate that it was employed as a means of raising the fluid pressure within the cœlom. In the next stage of development that part of the body cavity which contained the orifices of the renal tubules and the glomeruli became largely constricted off from the rest, and by means of imperfect septa the glomeruli also became partially separated from one another. This indicates that the function of the glomerulus has now been restricted almost solely to work in association with renal excretion. Later, this becomes entirely the case by the complete separation of that portion of the cœlom from the rest. Each glomerulus then works in conjunction with a renal tubule, but at first the number of the latter is largely in excess of the former. The material secreted at the glomerular surface is now conducted entirely to the tubule, as is also any formed by the isolated portion of the cœlomic endothelium. It is very significant that as soon as the relationship between glomerulus and tubule is completed the latter loses its cilia, only the cells of the neck of the tubule retaining them in some animals. This indicates that some other mechanism for the propulsion of fluid down the tubule has taken the place of the ciliary movement. This, according to my view, is the propulsive action of the glomerular capillary loops.

Previous Work Bearing upon the Subject.

L. Hill, in discussing the general distribution of pressure through a soft and yielding animal tissue, arrives at the conclusion that filtration is an impossible mechanism at the glomerular surface. With much that Hill expresses in his paper on "Filtration in the Living Organism,"* I am in complete agreement, but in several points I think he is incorrrect. Thus, he considers that the glomerular capillary pressure must be transmitted in undiminished amount throughout the whole renal tissue. This implies that the wall of Bowman's capsule is incapable of offering any resistance to extension, and similarly, too, for the walls of the tubule. Our measurements show, however, that while these structures expand, they offer resistance to expansion. They indicate that a higher pressure has been acting on the internal surface of the tubule than on the outer, and especially until a sufficient dilatation has been produced to make the kidney substance as a whole expand, and thus render the Capsule tense. From that point on, the tension in the kidney substance rapidly rises. I have found by measurements of the blood flow that at this point the blood flow falls, due, that is, to compression of the capillaries around the convoluted tubules and of the renal veins. The fact that the capillary system which originates this pressure consists of characteristic tufts which lie entirely within capsules is very significant. In certain forms of tubular nephritis, in which the tubules are blocked or obliterated, and have been so for a considerable time, the capsules are often found distended to a volume even ten times greater than the normal volume. In these cases the glomerulus is collapsed and shrunken to a minute structure, which appears as a mere projection into the swollen capsules.

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* 'Biochem. Journ.,' 1906, vol. 1, p. 55.

In my opinion, too, Hill does not allow a sufficient fall in pressure-head between the glomerular capillaries and the tubule capillaries. The efferent blood-vessel of the glomerulus is of small diameter and fairly long. Hence with the exceedingly rapid blood flow observed during diuresis, there must of necessity be a considerable pressure difference between these two capillary systems. I cannot, therefore, agree with Hill's statement: "The pressure of the secretion cannot be normally greater than the pressure *in* the veins, for otherwise the secretory pressure would compress the veins"; nor, again, with the statement: "The secretion moves onward, I take it, by phenomena of adsorption."

At about the same time Filehne and Biberfeld* reasoned that filtration at the glomerular surface was an impossibility, since there were no firm supporting structures capable of resisting any pressure. They, too, consider that the glomerular capillary pressure is at once transmitted through the whole renal substance, leaving no pressure difference available for filtration through the glomerular surface. While agreeing with them that but a very minute pressure difference can exist between the glomerular blood-pressure and the pressure of the secreted fluid within Bowman's capsule, I am in disaccordance with them, for reasons already stated, in their idea that the glomerular pressure is at once transmitted in undiminished amount to the general renal substance.

Shortly after I had expressed my views as to the work of the glomerulus, Lamy and Mayer† published a paper in which they suggested that the glomerulus by its pulsation acted as a kind of heart, and by its piston-like movements drove the liquid forward in the tubule, and favoured its discharge by overcoming the friction and the capillarity of the tubule. They do not consider that the glomerulus plays any important part in the secretion of water. If it secretes any at all, this is in their opinion quite a minor rôle. According to them the glomerulus performs mechanical work solely by virtue of its pulsation, and consequently their view differs widely from mine. I am, in the first place, in wide disagreement with them in that I consider that the main bulk of the water is secreted by the glomerular surface. There is abundant evidence to prove this. I need only refer to the work of Miss Cullis upon secretion in the frog's kidney,* or to the results I have briefly described above upon secretion in the rabbit's kidney after division of the spinal cord. As is seen from what I have stated, the fact that the glomerulus pulsates has but little bearing, if any, upon its work in propelling the secreted water along the tubule. That pulsation is unimportant in the propulsor action of the glomerulus is borne out by the fact that the urine flows quite freely along the ureter of an excised kidney perfused with fluid at constant pressure, and if in these cases the perfusing fluid be of correct composition, the kidney presents at the end of the experiment appearances exactly comparable to those found by Mackenzie and myself after active diuresis in the intact animal. It is possible that pulsation may play a part in producing the primary dilatation of the convoluted tubule. In an artificial schema representing the glomerulus and tubule, I have found that the volume of fluid driven along the capillary tube by a pressure made to vary in imitation of the pulse variations is exactly the same as if a steady pressure at the mean height of the varying one is used. This indeed was to be expected from theoretical reasons. The value of a varying pressure only arises when the tubule along which the fluid is to be driven has first of all to be expanded.

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* 'Pflüger's Archiv,' 1906, vol. 111, p. 1.

† 'Journ. de Physiol.,' 1906, vol. 7, p. 660.

In conclusion, then, we may summarise what I have said in the following way:—

The glomerulus is a secreting surface whose chief function is to secrete the main bulk of the water of the urine, but it is also thrown into activity by such substances as salts, urea, dextrose and caffeine. Its highly characteristic shape is to enable it to act as a means of setting up a pressure-head sufficient in amount to drive the secreted water down the long urinary tubule. The pressure originating from this is also transmitted in some degree through Bowman's capsule to the general tissues of the cortex, thereby exerting a pressure upon the external surfaces of all the tubules lying in the cortex. To what degree the pressure on the external surfaces of the convoluted and other tubules lies below the glomerular capillary pressure I am not yet able to state definitely. The fact that the convoluted tubules show such marked evidences of having been subjected to a high internal pressure certainly indicates a considerable diminution. I have also given reasons for believing that the general pressure conditions so typical of the cortex are non-existent in the medulla; there, apparently, the internal pressure acts upon the loops of Henle in undiminished amount, and must be supported either by the basement membrane of those tubules, or by the general tissue of the medulla itself. At present the former seems the more probable. Lastly I have given evidence attained by the application of yet another method, which enables us to determine from histological evidence the part of the urinary apparatus thrown into activity by the different urine exciting substances.

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* 'Journ. of Physiol.,' 1906, vol. 34, p. 250.

[*Addendum.*—Shortly after I delivered this lecture before the Royal Society, letters appeared in the 'Lancet' and the 'British Medical Journal' by Mr. Wm. Woods Smyth, claiming that his brother, Dr. A. W. Smyth, had over 30 years ago anticipated the views I now expressed. Dr. Smyth's views of the function of the kidney appeared in a pamphlet by Mr. John Gamgee, in the 'New Orleans Medical and Surgical Journal' for May, 1880, and were based upon microscopic examination of the kidney, and upon the fact that the kidney pulsated with each heart-beat. As far as I am aware, no reference to his views has ever appeared in the literature upon the kidney. They concerned the glomerulus and the circulation through the kidney. He denies the existence of any "connection between the capsule of the Malpighian body and the interior of a uriniferous tubule," and also "having observed that the hyaline membrane, enclosing each glomerule, was unprovided with epithelium, essential to every secreting structure, Dr. Smyth perceived that so delicate a sac would rupture, and the plexus be destroyed, if subjected to hydrostatic pressure, either during secretion or from accidental regurgitation." But the main point in relation to this lecture is his view of the mode of working of the glomerulus. This he describes in the following terms:—"Every heart-beat is attended by turgescence of the glomerule. The loops, from their position and form, must swell outward and inward in all directions, and, constricting the efferent vessel, momentarily impede the blood's exit. At each cardiac diastole, the arterial column sustaining the blood in its channel, the Malpighian loops recoil and fill the current in the secreting vascular rete. And this is Dr. Smyth's view of the special function of the Malpighian bodies. Their alternate turgescence constituting a 'rhythmic vascular impulse,' a uniform, safe, and sufficient expelling pressure is maintained on the coiled tubes, and, indeed, on the whole excreting structure of the kidney. Those acquainted with the laws which govern the flow of liquids can readily understand that the power required to maintain a circulation, beyond the coils of the glomerule, would be destroyed, if a mere physical transudation could occur through the loops, so well disposed to bring the very active pulsation to bear on the maintenance of a circulation."

"The unmistakable constriction of the efferent vessel, on the filling of each glomerule, causes an alternation between clearance of the tubuli and the flow of blood in the secreting vascular rete. The glomerules are filled during the heart's systole; the secreting rete is turgid during the heart's diastole."

Undoubtedly Dr. Smyth's conjecture was in the right direction, but his erroneous conclusion that Bowman's capsule did not open into the tubule, and the fact that he ascribed all the expelling power of the glomerulus to its pulsation, will indicate sufficiently the great divergence of his views from those I have expressed in my lecture.]

DESCRIPTION OF PLATE.

Fig. 1.—Microphotograph of Cortex of Kidney of Cat, after period of rest, showing absence of lumen in convoluted tubules and irregular outline of glomeruli. ×120.

Fig. 2.—Microphotograph of Cortex of Kidney of Cat, after sulphate diuresis, showing widely dilated tubules and distended capsules, which are now rounded and contain much fluid. The glomeruli are larger than in the resting kidney, but not filling the capsules. ×120.

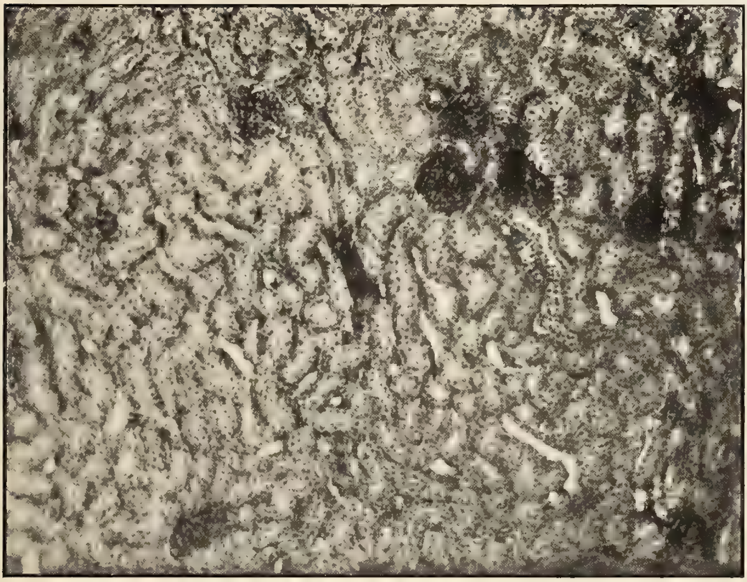

FIG. 1.

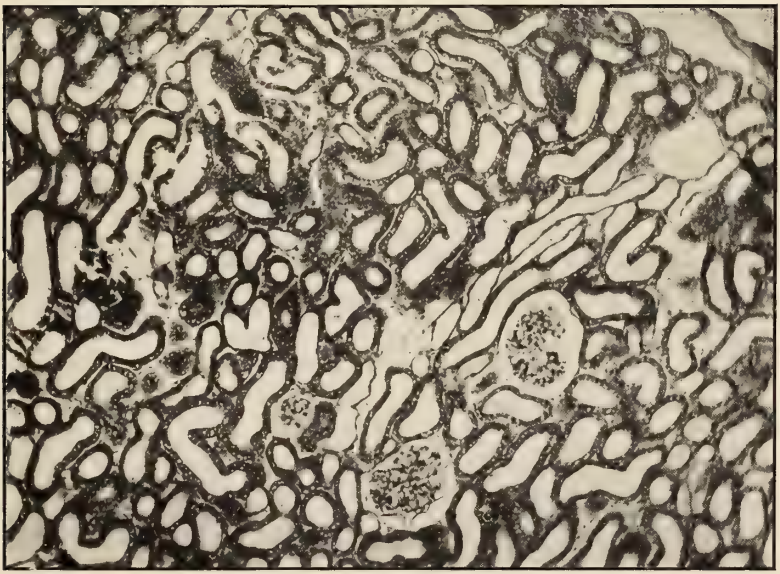

FIG. 2.

On Changes in the Glomeruli and Tubules of the Kidney accompanying Activity.

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(Received December 9, 1912,—Read February 20, 1913.)

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[Plate 27.]

The experiments described in this paper were designed to test the correctness of the view put forward by one of us,* namely, that the glomerulus is a propulsor. If this view be correct, the marked dilatation of the tubules, which is so prominent a feature in a kidney after active diuresis, is simply the expression of the forcible distension of the tubule from within, effected by the discharge of fluid from the glomerulus down the tubule, the active propelling and dilating force being the intraglomerular blood-pressure transmitted through the glomerular capillary cells and epithelium. As, however, the condition of the glomerulus after active secretion has not been made the subject of extensive observation, it seemed probable that a thorough study of the alterations in size and appearance of both tubule and glomerulus might give many points of importance in criticising the propulsion theory. Thus, if the capsule be free to expand, we may find it enlarged after active diuresis; and again, if the propulsive action of the glomerulus is complete and instantaneous, we should find the glomerulus filling Bowman's capsule completely under all conditions. But it was also possible that, after a very free secretion of water, there might be a considerable accumulation of fluid between the glomerulus and the capsule wall. We therefore measured the sizes of the capsules, the glomeruli and the tubules in kidneys, before and after diuresis had been set up under varying conditions. The more important of these states were:—

1. The kidney at rest.
2. The kidney secreting freely. This we term an "active free" kidney.
3. Decapsulated and secreting freely. This we term an "active decapsulated" kidney. The aim of the procedure was to test the explanation offered by the theory as to the meaning of the Capsule.†

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* *Vide* Croonian Lecture, *supra.*

† As in the course of this paper we shall be referring constantly to the Capsule of the kidney and to Bowman's capsule, we will, in order to avoid needless repetition, distinguish between them by employing a capital letter whenever we refer to the former.

4. With the ureter ligatured. This we term an "active obstructed" kidney.

We soon found that the different parts of the renal tubule, and more especially of Bowman's capsule and the glomerulus, varied considerably in size in different animals, so that it is necessary in making comparisons to use only, in the first instance, opposite kidneys in the same animal. Hence, our series of experiments comprises each possible combination in the above-named types of experiments.

All our experiments were performed upon cats anæsthetised with a mixture of chloroform and ether.

In all experiments, the kidney was removed and fixed in the following way. It was first carefully freed from subperitoneal fat, and a ligature then tied tightly around the pedicle close to the hilum. A second ligature was next tied around the pedicle a little nearer the aorta, and the pedicle divided between the ligatures. The object of ligaturing the pedicle was to keep the urine within the tubules, and as far as possible in the position it occupied at the instant of ligature. The kidney was dropped intact into a beaker of 20-per-cent. formalin made up with 0·9 per cent. NaCl. The beaker and solution had previously been weighed, and it was now weighed a second time, giving the weight of the kidney. At the end of an hour, the kidney was sliced into thin sections, fixation in formalin completed, and the pieces imbedded and sections prepared. The following measurements were then taken:—

1. An equatorial diameter of the capsule at right angles to the polar diameter.

2. The polar diameter, *i.e.* one passing through the point of entrance of the blood-vessels.

3. The greatest distance between the glomerulus and the capsule if the two were not in contact.

4. The maximal diameter of a typical proximal convoluted tubule.

5. The diameter of its lumen.

6 and 7. Similar measurements of a typical section of the distal convoluted tubule.

The glomeruli measured were taken at random, care being exercised only to measure those in which the section passed centrally. This was generally fairly easy to attain by taking those which showed the point of entrance of the blood-vessel into the glomerulus. From these measurements, calculations were made of the approximate volumes of the capsule and the glomerulus respectively. To obtain these, we regarded the capsule as equal in volume to a sphere whose diameter was the mean of the two diameters of the capsule. The figures representing volumes given in this paper were obtained by cubing the mean radius of the capsule expressed in microns, and dividing it by 1000. Hence, to convert the figures into cubic millimetres, they must be multiplied by $4{\cdot}2 \times 10^{-6}$. The glomerulus was also compared to a sphere, whose diameter was the diameter of the capsule minus the maximum space between the glomerular surface and the capsular surface. The difference between the two volumes thus ascertained gives us an approximate estimate of the volume of the fluid contained within the capsule.

In measuring the tubules a section of a proximal convoluted tubule lying near to the glomerulus was selected, and that section of the distal convoluted tubule which lies close to the point of entrance of the vessels into the glomerulus. Hence the proximal tubule probably belonged to the glomerulus measured, and the distal tubule certainly did so belong.

I. *Comparison between a Resting and an Active Kidney.*

A. *The Glomerulus and Capsule.*—There are always marked differences between a resting and an active glomerulus. A resting glomerulus appears to be made up of a dense tissue closely packed with nuclei (fig. 1). The glomerular surface always lies in contact with the capsule wall, and the whole structure is usually irregularly quadrangular in outline. After activity the glomerulus stands away clearly from the capsule. The outline of the glomerulus is lobular, and in structure it is much looser than the resting glomerulus (fig. 2). It also appears to be filled with large vacuole-like spaces approximately circular in section. The nuclei are well separated. As a rule the number of blood corpuscles contained in the glomerular vessels is quite small, far fewer than in the resting glomerulus. This we think may be due to the expulsion of the blood from the capillary loops after excision of the kidney, or to *post-mortem* laking of the corpuscles. The latter may be produced by the diffusion of water from the capsule through the walls of the capillary loops after the epithelial cells have died, and before the fixative has had time to act upon them. This would account for the very characteristic vacuolated appearance of the glomeruli already alluded to.

We were never able to keep the blood in a kidney that was excised at the height of activity. At the instant of excision such a kidney is hard and tense, and instantly becomes soft when the first ligature is tied round the pedicle. This is even the case though the vein be first ligatured, and though the kidney may have been separated from its surrounding tissues before the diuretic was administered in order to give ample time for closure of the many small vessels passing through the Capsule. Even then there is a distinct escape of blood through the Capsule, and the cortex rapidly pales in colour as the tension falls. The greater the tension at the instant of ligature, the greater is this paling of the cortex, and the sections of such kidneys may show but traces of blood in any of the capillaries, and but little in the veins.

The change in the shape of Bowman's capsule when the kidney becomes active is very distinctive. It becomes circular or elliptic in section, and there is always fluid between the glomerulus and the capsule wall. In many instances we have noted one other highly suggestive appearance. This is that the first portion of the proximal tubule has, in cases in which a free diuresis was established, been distended so as to appear almost a part of the capsule wall. An instance of this is illustrated in fig. 3. It is a very clear indication that the capsule and the first part of the convoluted tubule have been subjected to a high internal pressure. There are further indications, moreover, that the capsule has been distended to a size much larger than it appears in the section after fixation. The action of a high intracapsular pressure also adequately explains the change of shape from irregularly quadrangular to spheroidal or ellipsoidal.

B. *The Tubules.*—The contrast between the tubules at rest and after they have been in activity is just as striking, and in some particulars has already been described by several observers. In this paper we deal entirely with changes in the total diameter and in the lumen of the tubules, and, moreover, restrict our attention for the most part to the two convoluted tubules.

The magnitude of these several changes is brought out by the following measurements taken from Experiment 10. The measurements are in microns, and each is the mean of 10 measurements:—

Expt. 10.—R. kidney resting. L. kidney free.

	R. μ.	L. μ.
Glomeruli and capsules—		
Equatorial diameter	108·4	144·0
Polar diameter	78·4	103·6
Space	3·0	23·8
Hence		
Mean diameter capsule	93·4	123·8
" " glomerulus	90·4	100·0
Approximate volume capsule	102	237
" " glomerulus	92	125
" " fluid	10	112

Convoluted tubules—			
Proximal.	External diameter ...	41·4	41·4
	Lumen	0·0	17·6
Distal.	External diameter ...	21·2	32·4
	Lumen	7·2	20·6

	grm.
Weight of R. kidney	10·9
„ L. kidney	16·2

These figures show most clearly how extensive a change in size of the different parts of the renal tubule occurs when it is thrown into activity. Thus the capacity of the capsule is more than doubled (to 232 per cent.), chiefly because of the very large accumulation of fluid which has been secreted. The glomerulus is, however, increased to 136 per cent. of the volume of the glomerulus at rest. The differences are in reality still more marked, for a glomerulus actually at rest has no space between the glomerulus and the capsule wall, whereas in the right kidney of this animal no less than 7 of the 10 capsules measured contained fluid, though but small in amount.

We may conclude, then, that both Bowman's capsule and the glomerulus are distensible structures, and, further, that during activity the glomerulus does not remain in contact with the capsule wall, all of which strongly opposes the filtration theory of glomerular activity. These two conclusions are confirmed by every experiment we have performed.

When we turn to the measurements of the tubules the changes are equally striking. The external diameter of the proximal tubule is usually unaltered, but, whereas the resting tubule has no lumen, the tubule after action has a large lumen (43 per cent. of the total diameter). With the distal convoluted tubule the case is somewhat different. The total diameter is markedly increased (to 153 per cent.). The lumen of the resting tubule is 34 per cent., but that of the active tubule 64 per cent. of the total diameter of the tubule. Also, the lumen of the active tubule is 2·86 times greater than that of the resting. Apparently, then, the basement membrane of the proximal convoluted tubule is practically inextensible with the forces at play in this instance, whereas that of the distal convoluted tubule is extensible. In both tubules the cells are distinctly flattened against the basement membrane as a result of activity.

II. *Comparison between a Resting and a Decapsulated Kidney.*

The measurements obtained in an experiment of this character (Experiment 11) were as follows:—

Expt. 11.—R. kidney, resting. L. kidney, decapsulated and secreting freely.

	R.	L.
	μ.	μ.
Glomeruli and capsules—		
Equatorial diameter	100·4	112·0
Polar diameter	73·6	95·2
Space	3·0	14·6
Hence		
Mean diameter capsule	87·0	103·6
„ „ glomerulus	84·0	89·0
Approximate volume capsule	82	139
„ „ glomerulus	74	88
„ „ fluid	8	51
Convoluted tubules—		
Proximal. External diameter	46·0	42·0
Lumen	1·4	19·4
Distal. External diameter	24·0	28·0
Lumen	10·8	17·6

	grm.
Weight of R. kidney	8·4
„ L. kidney	10·6

In this experiment the changes are entirely in the same direction as in the preceding, and the magnitude of the various changes is also approximately the same. If anything, the free kidney in the preceding experiment showed rather greater changes in comparison to the resting than did the decapsulated kidney of this experiment. The difference is, however, accounted for by the fact that the diuresis in Experiment 10 was greater than in Experiment 11.

The increase in volume of the capsule is to 170 per cent., of the glomerulus to 119 per cent. One notable difference is that in this experiment the external diameter of the proximal convoluted tubule was less after diuresis than when at rest.

III. *Comparison of a Free Kidney with a Free Decapsulated Kidney.*

Expt. 3.—R. kidney free. L. kidney free and decapsulated.

	R.	L.
	μ.	μ.
Glomeruli and capsules—		
Equatorial diameter	135·2	142·4
Polar diameter	100·8	112·0
Space	15·2	20·6

Hence

Mean diameter capsule	118·0	127·1
„ „ glomerulus	100·8	106·5
Approximate volume capsule......	205	257
„ „ glomerulus	128	151
„ „ fluid	77	106

Convoluted tubules—

Proximal.	External diameter ...	44·8	48·0
	Lumen	13·0	19·8
Distal.	External diameter ...	33·2	39·2
	Lumen	24·8	29·2

	grm.
Weight of R. kidney.........	20·6
„ L. kidney.........	19·1

The two kidneys show the general changes of a diuresis in a well-marked manner. The experiment further shows that the effect of decapsulation is to cause a relatively greater expansion of both capsule and glomerulus. Also, the capsule is not so well emptied as in the normally active kidney. The difference in the dilatation of the convoluted tubules is again in favour of the decapsulated kidney. This is particularly seen with regard to the lumen of the proximal convoluted tubule. Whereas the ratio of the external diameter of the first convoluted tubule of the decapsulated kidney to that of the free kidney is 1 to 1·07, the ratio of the lumina is 1 to 1·53.

Hence we may conclude that decapsulation results in an increased distension of all the cortical parts of the kidney tubule when it is thrown into activity.

In the next group of experiments one of the kidneys was obstructed. The group comprises three comparisons.

IV. *Comparison of a Resting Kidney with an Obstructed Kidney.*

Expt. 12.—R. kidney resting. L. kidney obstructed.

	R.	L.
	μ.	μ.
Glomeruli and capsules—		
Equatorial diameter	98·4	130·4
Polar diameter	76·0	111·2
Space	1·2	24·8

Hence

Mean diameter capsule	87·2	120·8
„ „ glomerulus	86·0	96·0
Approximate volume capsule......	83	220
„ „ glomerulus	80	111
„ „ fluid.........	3	109

Convoluted tubules—

Proximal.	External diameter ...	44·0	42·8
	Lumen..................	0·0	19·4
Distal.	External diameter ...	25·4	31·8
	Lumen..................	11·0	21·8

	grm.
Weight of R. kidney	7·7
„ L. kidney	10·9

The general changes are in the same direction as before. Perhaps the most marked difference between this and the previous kidneys examined is the large volume of fluid contained within the capsule, and the relatively small size of the glomerulus. Again, we note that there is no change in the external diameter of the proximal convoluted tubule, whereas the distal is extended to 125 per cent. of its resting diameter. As illustrated by the lumina, a very considerable volume of urine is collected within the tubules, particularly in the distal tubule.

V. *Comparison of a Free Kidney with an Obstructed Kidney.*

Expt. 6.—L. kidney free. R. kidney obstructed.

	R. μ.	L. μ.
Glomeruli and capsules—		
Equatorial diameter	99·6	110·8
Polar diameter	77·2	100·0
Space	6·2	16·0

Hence

Mean diameter capsule	88·4	105·4
„ „ glomerulus	82·2	89·4
Approximate volume capsule......	86	146
„ „ glomerulus	69	89
„ „ fluid.........	17	57

Convoluted tubules—			
Proximal.	External diameter	39·2	39·4
	Lumen	6·6	14·0
Distal.	External diameter	22·8	28·2
	Lumen	12·6	18·4

	grm.
Weight of L. kidney	13·5
„ R. kidney	15·5

This experiment shows quite clearly the great effect of obstruction upon the distension of the capsule and accumulation of fluid within the capsule. Obstruction also causes a distinct further dilatation of the distal convoluted tubule, and an increase in the lumina of both parts of the tubule.

VI. *Comparison of a Free Decapsulated Kidney with an Obstructed Kidney.*

Expt. 7.—R. kidney decapsulated. L. kidney obstructed.

		R. μ.	L. μ.
Glomeruli and capsules—			
	Equatorial diameter	121·2	130·4
	Polar diameter	102·0	111·2
	Space	9·8	20·0
Hence			
	Mean diameter capsule	111·6	120·8
	„ „ glomerulus	101·8	100·8
	Approximate volume capsule	174	220
	„ „ glomerulus	132	128
	„ „ fluid	42	92
Convoluted tubules—			
Proximal.	External diameter	42·0	41·4
	Lumen	15·4	17·6
Distal.	External diameter	28·6	31·0
	Lumen	20·4	21·2

	grm.
Weight of R. kidney	10·7
„ L. kidney	11·0

The results of the measurements in this experiment show that obstruction of the ureter results in an increased expansion of the capsule of the obstructed, as compared to that of the free active kidney; this is entirely due to a greater accumulation of fluid within it. The convoluted tubules show corresponding differences. The effect as before is mainly felt in the distal tubule, which shows a somewhat greater expansion. The lumina in the proximal tubules are greater in the obstructed kidney than in the free kidney. In this experiment the blood-pressure was rather low, but the diuresis good.

In all these obstructed kidneys the effect upon the medulla is very marked. Not only is the pelvis of the kidney greatly distended, but the pyramid is driven back towards the cortex, and appears very much shrunken. We have often seen it so contracted as to appear only about a quarter or less of its normal size. In the sections the collecting tubules are flattened and empty, the loops of Henle, however, contain fluid, and often appear to be about the same size as in the normal active kidney. The appearance of the pyramids is so characteristic that one can at once decide whether or no the ureter of that kidney had been obstructed in the experiment.

The last group of experiments comprises a comparison of various kidneys with a kidney which was both obstructed and decapsulated.

VII. *Comparison of a Resting Kidney with a Decapsulated and Obstructed Kidney.*

Expt. 13.—R. kidney resting. L. kidney decapsulated and obstructed.

	R.	L.
	μ.	μ.
Glomeruli and capsules—		
Equatorial diameter	110·4	128·0
Polar diameter	79·6	110·8
Space	3·4	21·2
Hence		
Mean diameter capsule	95·0	119·4
„ „ glomerulus	91·6	98·2
Approximate volume capsule	107	213
„ „ glomerulus	96	118
„ „ fluid	11	95
Convoluted tubules—		
Proximal. External diameter	46·0	49·6
Lumen	0·0	26·4
Distal. External diameter	21·8	34·4
Lumen	10·6	24·4

	grm.
Weight of R. kidney	14·5
„ L. kidney	19·3

An examination of the figures brings out an enormous increase in the size of the capsules, due chiefly to the increase in the amount of the fluid contained. The effect upon the convoluted tubules is again most marked. Otherwise the figures require no further comment.

The right kidney was not completely at rest, as was indicated by the microscopic appearance of the glomeruli. In every instance there was fluid between the glomerulus and the capsule.

VIII. *Comparison of a Free Kidney with a Decapsulated and Obstructed Kidney.*

Expt. 1.—R. kidney free. L. kidney decapsulated and obstructed.

	R. μ.	L. μ.
Glomeruli and capsules—		
Equatorial diameter	135·6	143·6
Polar diameter	106·8	125·6
Space	23·8	31·6
Hence		
Mean diameter capsule	121·2	134·6
„ „ glomerulus	97·4	103·0
Approximate volume capsule	223	305
„ „ glomerulus	116	137
„ „ fluid	107	168
Convoluted tubules—		
Proximal. External diameter	48·2	51·4
Lumen	13·2	24·0
Distal. External diameter	38·6	39·8
Lumen	29·0	30·8

The general result of the experiment shows that the glomeruli and convoluted tubules are more distended in the decapsulated and obstructed kidney than in the free kidney. In this instance the volume of the capsules became enormous, with only a slight increase in the volume of the glomeruli. We would emphasise the very great size of the lumen of the proximal convoluted tubule.

Expt. 4.—R. kidney free. L. kidney decapsulated and obstructed.

	R. μ.	L. μ.
Glomeruli and capsules—		
Equatorial diameter	137·6	152·4
Polar diameter	112·4	122·4
Space	10·4	22·0

Hence

Mean diameter capsule		125·0	137·4
„ „ glomerulus		114·6	115·4
Approximate volume capsule		244	324
„ „ glomerulus		188	192
„ „ fluid		56	132
Convoluted tubules—			
Proximal.	External diameter	45·6	43·4
	Lumen	15·2	22·8
Distal.	External diameter	27·8	29·4
	Lumen	15·8	21·2

	grm.
Weight of R. kidney	16·5
„ L. kidney	18·7

The results obtained in this experiment in every way confirm those shown in the previous experiment.

IX. *Comparison of a Decapsulated Kidney with a Decapsulated and Obstructed Kidney.*

Expt. 2.—L. kidney decapsulated. R. kidney decapsulated and obstructed.

		L. μ.	R. μ.
Glomeruli and capsules—			
Equatorial diameter		142·4	161·2
Polar diameter		122·4	129·2
Space		6·2	12·4
Hence			
Mean diameter capsule		132·4	145·2
„ „ glomerulus		126·2	132·8
Approximate volume capsule		290	383
„ „ glomerulus		251	293
„ „ fluid		39	90
Convoluted tubules—			
Proximal.	External diameter	47·4	47·2
	Lumen	12·4	19·2
Distal.	External diameter	30·4	36·4
	Lumen	18·8	24·2

	grm.
Weight of L. kidney	13·6
„ R. kidney	16·6

The results of the experiment are again very decisive, a notable point being the large volume of the glomeruli in both kidneys. We would again point out that the main effect upon the convoluted tubules is seen in the distal tubules.

Expt. 5.—R. kidney decapsulated. L. kidney decapsulated and obstructed.

	R. μ.	L. μ.
Glomeruli and capsules—		
Equatorial diameter	144·8	151·6
Polar diameter	111·6	136·8
Space	24·0	37·6
Hence		
Mean diameter capsule	128·2	144·2
„ „ glomerulus	104·2	106·6
Approximate volume capsule	263	375
„ „ glomerulus	141	151
„ „ fluid	122	224
Convoluted tubules—		
Proximal. External diameter	45·6	51·0
Lumen	20·8	25·2
Distal. External diameter	34·0	42·0
Lumen	23·0	30·6

	grm.
Weight of R. kidney	17·6
„ L. kidney	18·0

The figures are in agreement with those of the preceding experiment, with the exception that the volume of the glomeruli in this instance is small. In Experiment 2 the blood-pressure was low (83 mm. Hg) and the diuresis moderate, while in Experiment 5 the blood-pressure was high (130 mm. Hg) and the flow of urine rapid.

X. *Comparison of an Obstructed Kidney with a Decapsulated and Obstructed Kidney.*

Expt. 8.—L. kidney obstructed. R. kidney decapsulated and obstructed.

	L. μ.	R. μ.
Glomeruli and capsules—		
Equatorial diameter	109·2	108·0
Polar diameter	95·6	95·2
Space	11·8	9·2

Hence

Mean diameter capsule		102·4	101·6
„ „ glomerulus		90·6	92·4
Approximate volume capsule		134	131
„ „ glomerulus		93	99
„ „ fluid		41	32
Convoluted tubules—			
Proximal.	External diameter	40·4	41·4
	Lumen	17·8	18·2
Distal.	External diameter	25·4	29·6
	Lumen	17·2	21·0

	grm.
Weight of R. kidney	15·8
„ L. kidney	15·8

In this experiment the blood-pressure was low and the flow of urine small, and with it again the volume of fluid in the capsule is small. In general, it confirms the result of the preceding experiments. Decapsulation combined with obstruction produces a greater distension of the tubules than obstruction alone. With a more abundant diuresis than occurred in this experiment a similar result is found in the capsules and glomeruli.

In the following tables we collect the results obtained in our thirteen experiments. In the first we give the means of the approximate volumes of Bowman's capsule, glomerulus and fluid, and in the second the ratios of these to the similar structures in the resting kidney.

We would not lay much stress upon comparisons between these figures, except when the differences are very marked. There are so many varying factors upon which the actual magnitudes of the measurements depend that to do so would lead to erroneous conclusions. Thus, the results vary with the blood-pressure, with the degree of diuresis established, the duration of the diuresis, and especially with the degree of extensibility of the kidney Capsule, and of the general renal tissues, both of which we know to vary greatly in different animals. Table II, however, shows very decisively the enormous changes in size of the glomerulus and capsule caused by active secretion of water, and more especially in the very great accumulation of water within the capsule during activity. All these results are of the highest importance in disproving the possibility of filtration at the glomerular surface.

Table I.

	Volume Bowman's capsule.	Volume glomerulus.	Volume fluid.	No. of experiments.
Resting	94	85	9	4
Active free	227	137	90	4
„ decapsulated	229	162	67	5
„ obstructed	196	136	60	3
„ decapsulated and obstructed	277	157	120	6

Table II.—Ratios.

	Bowman's capsule.	Glomerulus.	Fluid.
Resting	1·00	1·00	1·00
Active free	2·42	1·61	10·00
„ decapsulated	2·44	1·91	7·44
„ obstructed	2·09	1·60	6·67
„ decapsulated and obstructed	2·95	1·85	13·34

In Tables III and IV we give similar figures for the convoluted tubules.

Table III.

	Proximal.		Distal.	
	External diameter.	Lumen.	External diameter.	Lumen.
Resting	44·4	0·4	23·4	9·9
Active free	45·0	14·8	33·0	22·6
„ decapsulated	46·0	17·3	33·2	22·2
„ obstructed	42·0	19·0	29·3	20·3
„ decapsulated and obstructed	47·3	22·6	35·3	25·4

Table IV.—Ratios.

	Proximal.		Distal.	
	External diameter.	Lumen.	External diameter.	Lumen.
Resting	1·00	1·00	1·00	1·00
Active free	1·01	37·00	1·41	2·28
„ decapsulated	1·04	43·25	1·42	2·24
„ obstructed	0·95	47·50	1·25	2·05
„ decapsulated and obstructed	1·07	56·50	1·51	2·57

These two tables bring out the following points:—

(1) The external diameter of the proximal convoluted tubule does not change on activity;

(2) A large lumen is developed in this tubule during diuresis. It varies with the degree of diuresis, and is markedly increased by obstruction of the ureter. Taking the average of all our observations it amounts to nearly 40 per cent. of the total diameter of the tubule;

(3) The distal convoluted tubule is expanded considerably (from 140 to 150 per cent. of its mean at rest); and

(4) The lumen, of considerable size (42·3 per cent. of the total diameter) even in a resting kidney, is more than doubled, and becomes 69·2 per cent. of the total diameter.

We may conclude, then, that the first convoluted tubule, *i.e.* that portion which is subjected to the highest internal pressure, is relatively inextensible transversely. The second convoluted tubule, on the other hand, is transversely extensible. From a further examination of our sections, we judge that the proximal convoluted tubules do indicate an extension in the longitudinal direction, but our present methods do not allow us to state this decisively.* All the results indicate that an internal pressure has existed during diuresis.

Conclusions.

Measurements of the diameters of the various portions of the renal tubule in the cat, when at rest and after diuresis under various conditions, show that Bowman's capsule, the glomerulus, and the second convoluted tubule are extensible structures, and are expanded during diuresis. The glomerulus leaves the capsule wall, a considerable accumulation of secretion being found between them. The lumina of all parts of the tubule become greatly enlarged.

All the appearances found are explained as resulting from the action of a high pressure in the fluid secreted by the glomerular epithelium, and are all in accordance with the propulsor theory of the action of the glomerulus.

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* If we may make the assumption that the volume of the cells of the convoluted tubule does not alter during diuresis, then the magnitude of the surface areas of the cells in a transverse section of the tubule gives us an indication of any change in length. If, for this purpose, we examine the results of Experiments 10, 11, 12, and 13, where we have direct comparisons of active with resting kidneys, we find that in all instances the proximal convoluted tubules are markedly stretched longitudinally. In Experiments 10 and 13 there is considerable shortening of the distal convoluted tubules, and in Experiments 11 and 12 slight shortening. In Experiments 10 and 13 the blood-pressure was high and the diuresis good. In Experiments 11 and 12 the blood-pressure was lower and the diuresis only moderate. Hence it would appear that, with a high internal pressure, this portion of the tubule is shortened, *i.e.* tends towards the spherical shape.

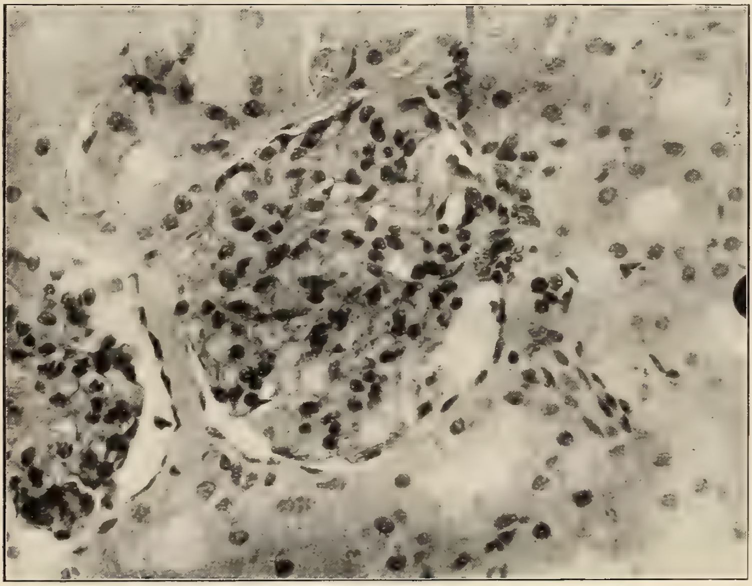

Fig. 1.

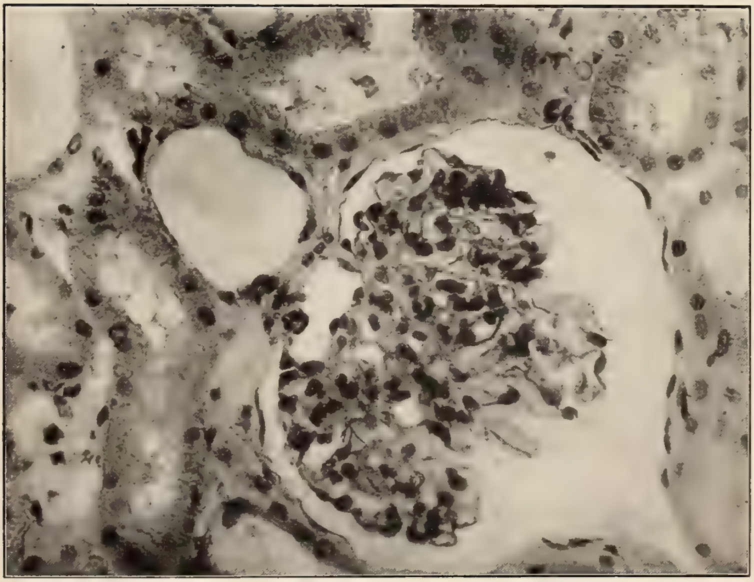

Fig. 2.

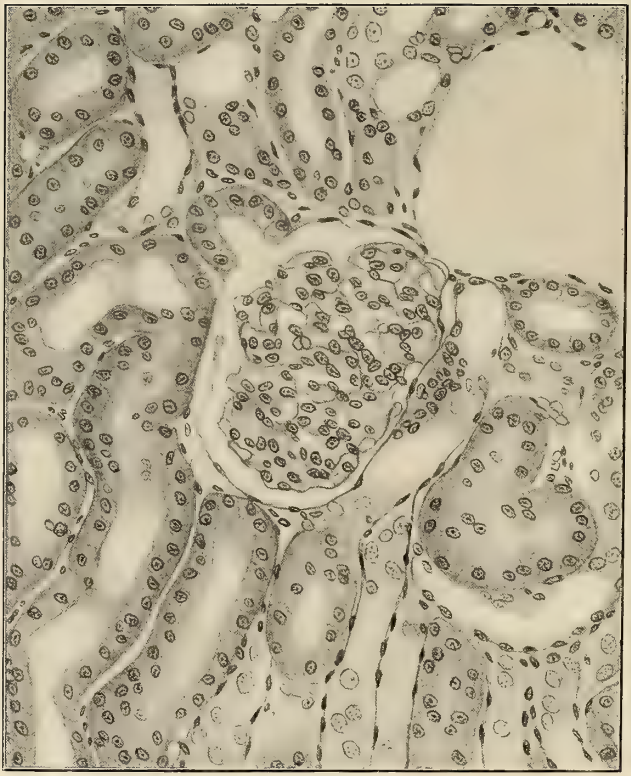

FIG. 3.

DESCRIPTION OF PLATE.

Fig. 1.—Microphotograph of Cortex of Dog's Kidney at Rest. × 500.
Fig. 2.—Microphotograph of Cortex of Opposite Kidney after Activity. × 500.
Fig. 3.—Cat's Kidney. Drawing of glomerulus and tubules after activity, showing dilatation of neck of tubule. × 500.

The Controlling Influence of Carbon Dioxide in the Maturation, Dormancy and Germination of Seeds.—Part II.

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Introduction.

In the first part of this paper the influence of carbon dioxide in inhibiting the germination of moist seeds was described. The results obtained are summarised on pp. 623–625 of this paper.

In the present paper the relation of this inhibitory effect of carbon dioxide to temperature and oxygen supply is first to be examined, and then will be studied further narcotic or inhibitory effects of CO_2 as exemplified in the natural inhibition of maturing seeds in the ovary, and the artificial prolongation of the dormant life of seeds which cannot survive naturally unless germination occurs soon after ripening.

Section I.—*The Relation to Temperature of the Inhibitory Effect of Carbon Dioxide on Germination.*

A large number of experiments were conducted to determine this relation. *Brassica alba* seeds were used. The result would appear to establish the conclusion that at low temperatures inhibition is caused by very small pressures of CO_2, while conversely at high temperatures high pressures of CO_2 are necessary to maintain continued dormancy. It would seem probable that this relation to temperature is significant in natural seasonal conditions. The technique in these experiments was the same as before described, the details of each experiment in full are unnecessary and a summary of the results obtained is given. The usual retardation effects were observed throughout, but the numbers in the table indicate only the final total germination out of 20 seeds.

Table I.—Total Number of Germinations with 20 *Brassica alba* Seeds in various Percentages of CO_2 in Air at different Temperatures. Compiled from 43 experiments.

Percentage of CO_2	0	2	4	6	9	12	15	18	24	30	36	42
3° C.	18	2	0									
7°	20	—	—	1	0							
10°	19	—	—	18	16	5	0					
17°	20	20	20	20	18	12	3	2	0			
20°	20	20	20	20	20	20	—	17	4	3	2	0
25′	20	20	20	20	20	20	20	20	19	19	14	7

The temperatures 10° C., 20° C., and 25° C. were maintained accurately within a variation of 0·5° C. The temperature of 3° C. obtained with melting ice varied to the extent of 1° C. The other two, 7° and 17° C., are averages of outdoor and indoor winter temperatures. The experiments were continued till no more seeds germinated.

It is necessary to consider the possibility of these results being due not to a decrease with rising temperature in the effectiveness of pressures of CO_2 in causing inhibition, but to an increase of oxygen stimulus caused by an increased permeability of the testa under the action of higher temperatures.

The following series of experiments were therefore conducted with *Brassica alba* seeds from which the testas had been carefully removed.

In these experiments with bare embryos, it was difficult in the early stages to tell by eye whether germination had begun or not. The bare embryos in the first stages of germination could not be differentiated, but had to be described as either all germinating or all not germinating. In order to bring the results given in the following Table more into relation with those formerly obtained, small figures have been inserted to express the relative condition of growth at the end of the experiment, and the delay in germination as compared with the controls.

Table II.—Total Number of Germinations with 20 Bare Embryos of *Brassica alba* Seeds in various Percentages of CO_2 at different Temperatures. Compiled from 14 experiments.

Percentages of CO_2	Control 0	5	10	20	30	40	50
5° C.	20	16	8				
16	20	20_{16}	20_{12}	20_{8}	20_{4}		
20	20	20_{18}	20_{16}	20_{12}	20_{8}	20_{4}	

Ten per cent. of oxygen was present in each case. The temperatures 16° C. and 20° C. were maintained accurately within a variation of 0·5° C. In the case of the experiments at 5° C. the temperature was less accurately controlled, being obtained by melting ice.

It will be seen from the foregoing Tables that a rise of temperature of 10° C. necessitates roughly the presence of three times as high a partial pressure of CO_2 to cause inhibition. Thus in Table I at 10° C. no germinations occurred with CO_2 pressures above 12 per cent., while at 20° C. germinations occur up to 36 per cent. Similarly at 7° C. no germinations occur above 6 per cent., while at 17° C. germination proceeds with pressures up to 18 per cent. It must be remembered that the actual partial pressures of CO_2 in the tissues of the embryos is probably higher, especially where the testa remains intact, than the values expressed in the tables for the partial pressures of CO_2 in the atmospheres used.

The result of this series of experiments, both with whole seeds and with bare embryos, thus clearly indicates that a rise in temperature necessitates an increase in the amount of CO_2 necessary to produce inhibition in the seeds of *Brassica alba.* Conversely, a fall in temperature reduces the necessary amount of CO_2 to cause inhibition.

This relation of carbon dioxide inhibition to temperature may be emphasised. In the case of drugs acting chemically on the protoplasm the expectation is that their action will be more effective at high than at low temperatures. Here with carbon dioxide the reverse result has been obtained. The fact must be borne in mind that we are dealing in this case with a gaseous agent more soluble at low than at high temperatures. This implies that to maintain in solution in the tissues the same concentration of carbon dioxide at a low temperature as at a high temperature necessitates a greater partial pressure of CO_2 in the atmosphere at the low temperature. Further work upon this relation of carbon dioxide inhibition in seeds to temperature is needed.*

Section II.—*Relation to Oxygen Pressure of the Inhibitory Effect of Carbon Dioxide on Germination.*

The presence of the testa between the embryo and its gaseous environment, as a membrane, only permeable with some difficulty, will, as has been pointed out, cause (1) a reduction in the amount of oxygen reaching the embryo, and (2) a relative rise in the actual CO_2 pressure in the embryo tissues.

It has been shown by the removal of the testa that temperature has, nevertheless, a direct effect in determining the inhibitory value of a given pressure of CO_2.

The following experiments were made to determine whether a varying oxygen supply might not also influence the inhibitory action of carbon dioxide. A large number of experiments were conducted at the same temperature, but with varying pressures of oxygen and carbon dioxide in the atmospheres used.

The testas were not removed in these cases. With a given pressure of CO_2, the temperature being fixed throughout, no variation in permeability in the testa was looked for. It is possible that an increased oxygen supply may cause a corresponding increase in the actual CO_2 pressure in the embryo tissues. The results show, however, that for the main purpose of the experiments this possibility may be neglected, as it clearly appears that an increase of oxygen supply decreases the inhibitory value of any given pressure of CO_2, while correspondingly a decrease in oxygen supply intensifies it, so that with small amounts of oxygen very low percentages of CO_2 will induce complete inhibition.

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* In a critical consideration of the actual pressures of CO_2 in the embryo tissues at any temperature we should have to take into account not only the external partial pressure of CO_2, but also the rate of CO_2 production in the tissues and the rate of the escape of this CO_2 from the tissues by diffusion. Roughly, in relation to different temperatures, these two processes tend to cancel one another, and their combined effect to give the same value at all temperatures. In the above experiments no account has, therefore, been taken of any change with temperature of the rate of CO_2 production in the tissues or of the rate of diffusion from the tissues.

Table III.—The Effect of Decreased Partial Pressures of Oxygen on Carbon Dioxide Inhibition in *Brassica alba* Seeds. Small amounts of CO_2 are sufficient to cause inhibition if little oxygen is present.

Atmosphere of oxygen, carbon dioxide, and nitrogen.		Germinations out of 20 seeds while in artificial atmospheres.				Proportion of remainder which finally germinated on removal to air.
Oxygen percentages.	Carbon dioxide percentages.	2nd day.	3rd day.	4th day.	5th day.	
per cent. 21 (air)	per cent. 0 (air)	18	20	20	20	
8	0	13	18	18	20	
8	1	13	19	19	20	
8	3	15	15	18	20	
8	6	—	12	13	16	All
4	0	6	18	18	19	All
4	1	—	1	1	3	All
4	6	—	—	—	0	All

Average temperature, 14° C.

It will be noticed from the preceding Table that the effect of decreasing the oxygen supply is to intensify the inhibitory action of carbon dioxide. Thus, with a decrease of oxygen to 8 per cent., the inhibitory effect produced by 6 per cent. of carbon dioxide is very marked. With a decrease of oxygen in the atmosphere to 4 per cent., complete inhibition is produced by 6 per cent. of carbon dioxide.

Here again it must be remarked that this relation may very likely be significant in many cases of delayed germination under the influence of CO_2 in natural conditions.

Table IV.—Effect of various Partial Pressures of Oxygen on the CO_2 Inhibition of Germination in *Brassica alba* Seeds. Total number of germinations obtained out of 20 seeds.

Percentages of CO_3	0	9	12	15	18	21	24	27	30
Oxygen 5 per cent.	20	6	4	0	0	0	0	0	0
„ 10 „	20	18	17	10	3	1	0	0	0
„ 15 „	20	20	20	15	10	2	0	0	0
„ 20 „	20	20	20	18	12	—	3	0	0
„ 30 „	20	20	20	20	—	11	5	2	1

Average temperature 16·7° C.; extremes 13–18° C. The atmospheric residuum is N_2 in these experiments.

It will be seen that the amount of oxygen present has a definite effect upon inhibition by CO_2. Where there is only a pressure of 5 per cent. oxygen, complete inhibition is obtained by 15 per cent. CO_2, but, with 30 per cent. oxygen present, as much as 30 per cent. CO_2 is scarcely sufficient at the temperature used to cause inhibition.

The result of these experiments, therefore, indicates that a rise in the partial pressure of oxygen within the limits experimented on necessitates an increase in the amount of CO_2 necessary to produce inhibition in the seeds of *Brassica alba*. Conversely, a fall in the partial pressure of oxygen reduces the necessary amount of CO_2 to cause inhibition.

Section III.—*Carbon Dioxide as a Factor in the Dormancy of the Maturing Seed on the Plant.*

(a) *Arrested Development of Maturing Seeds not due to Lack of Moisture—Retarding Influence of the Testa.*—The maturation of the seed in normal conditions has certain features upon which it is desirable to dwell briefly.

The growth of the embryo proceeds continuously after fertilisation. In some cases it quickly reaches an advanced stage, and the radicle, plumule, and cotyledons may be formed very early. This growth, moreover, appears to resemble in some respects the growth which takes place subsequently, after germination, but in others it has the appearance of partial inhibition, the radicle apparently being not free to sprout as in germination. This appearance of inhibition increases in the cases of most seeds, until at the stage of complete maturation growth is apparently arrested or suspended. That there is some restraining cause tending to prevent growth present in the seed during the series of changes which is producing maturation may be proved, as in the experiments following, by the fact that the embryo, often at a comparatively early stage, though the seed be far from ripe, can be caused to sprout if removed to air.

The following experiments were conducted in order to show that neither lack of water nor any physiological insufficiency in the embryo can be considered as the cause preventing the still maturing embryos of beans and peas from sprouting, and so becoming cases of viviparity:—

(1) Two lots, 10 peas and 10 beans, were taken from pods which were still perfectly green and hardly yet fully swelled. These two lots were set to germinate at 20° C. on damp sand, with the result that all the seeds germinated perfectly. From these experiments it is clear that, in the case of the bean (*Vicia faba*) and of the pea (*Pisum sativum*), for some considerable period before the natural drying process commences, and while the growth of the pods is continuing, the seeds, if removed and placed in

germinating conditions, are capable of immediate germination. In this and similar experiments it was noticeable, especially in the case of peas, that removal of the testa greatly increases the rate of this germination. The following experiment was typical:—

Table V.—Increased Rate of Germination in Maturing Seeds of Peas when Testa is removed.

Description of seed.	Germinations.		Remarks.
	3rd day.	8th day.	
Peas fresh from the pod (10 with testa)	0	4	The testas of the six not growing were removed on the 8th day. All these six then sprouted within two days.
Peas fresh from the pod (10 without testa)	3	8	

(2) Further experiments to test the power of the embryo of the ripening bean and pea, before drying has commenced, to grow without the addition of moisture, were necessary. To this end 10 bean embryos taken from seed in immature condition were placed in glass tubes closed at both ends with bored rubber corks. They were placed at such distances as to avoid contact with each other.

In six days the radicles of all had sprouted; similar results were obtained with embryos taken from immature pea seeds.

These experiments were repeated another year with confirmatory results.

The bare embryos germinate readily in the above conditions. In parallel experiments made with whole immature seeds, the presence of the testa still intact was found to retard sprouting constantly. This retarding effect of the testa was more marked in these cases where no water was added to the green seeds from the pods than in the experiments above, in which such seeds were germinated in the ordinary way on damp sand. In connection with this action of the testa it is of great interest to find that Guppy, in a recent book containing the results of a wide series of studies upon seeds, remarks that "it is noteworthy that the viviparous habit is associated with the absence of seed coats."

(3) Experiments with germinating beans after complete air-drying in the laboratory showed that, at the moment of sprouting, these seeds might actually contain less water than they did when originally removed from the pod. These experiments were conducted both with whole seeds and with

the embryo alone. The following are representative examples taken from a series of experiments:—

Table VI.—Showing that Beans germinated after Complete Air-drying may actually contain at the moment of sprouting less water than they did when originally removed from the fresh green pod in the last stages of maturation before drying on the plant had commenced.

	Original weight of 10 beans when removed from fresh pods before natural drying had commenced.	Weight of the same 10 beans at the moment of sprouting during germination on damp sand after complete air-drying.
	grm.	grm.
Whole seeds	27 ·7	22 ·6
,, ...	24 ·1	21 ·6
Embryos alone	22 ·0	20 ·1

From these experiments it would appear, therefore, that neither lack of water nor any physiological insufficiency in the embryo can be regarded as the factor limiting germination in the maturing seeds of peas and beans.

Finally, the action of the testa as a retarding influence on germination has to be noted. In addition to experiments already given with seed still immature, the following experiments were made with dried seeds:—

Table VII.—Retarding Influence of the Testa in Germination of Dried Seeds.

Description of seeds.	Water uptake after 24 hours in percentage of original dry weight.	Germinations.					
		1st day.	2nd day.	3rd day.	4th day.	5th day.	9th day.
Peas 8 days dried in air after removal from pod, temp. 18–14° C.—							
10 with testas	139	0	0	1	5		
10 without testas	112	0	0	5	7		
Dry beans—							
10 with testas	116	0	0	0	4	9	
10 without testas	119	0	1	3	8	10	
Dry beans—							
10 with testas	100	0	0	1	3	—	8
10 without testas	116	0	10	10	10	—	10

In the above Table the retarding influence of the testa in the germination of seeds after drying is well marked.

(b) *Direct Estimation of the* CO_2 *Content of Maturing and Germinating Seeds.*—An enquiry is strongly suggested as to how far the non-germination of the maturing seed, while still upon the parent plant, may be due directly to CO_2 inhibition or narcosis. In order to obtain evidence here an endeavour was made to ascertain the actual CO_2 content of ripening seeds. The method adopted was suggested by Dr. F. F. Blackman, for whose advice and direction during these researches I am deeply indebted. The technique of this method for determining the amount of CO_2 present in the tissues of seeds was as follows:—

Two lots of material of equal weight were taken in each experiment. One lot was crushed to thin paste in a mortar and left exposed to the air for 40 to 80 minutes. It seemed from experiments that this time was sufficient to allow the escape of the CO_2 present in the tissue mash. A known quantity of baryta was then added and a titration made with HCl. The second parallel lot was crushed immediately under an equal quantity of baryta and a similar titration made.

The difference between these two readings invariably showed that more baryta had been neutralised where the tissues had been crushed immediately in contact with it than where the tissue was first exposed for some time to air after crushing to a mash. These differences were taken as roughly expressing the relative CO_2 contents of the tissues used in these experiments.

The results obtained in a series of experiments made by this method to ascertain the CO_2 content of maturing peas and beans from fresh green pods and of the same seed during its drying in laboratory air, are given in the following table:—

Table VIII.—The CO_2 Content of Maturing Peas (*Pisum sativum*) and Beans (*Vicia faba*) when removed fresh from the Green Pod and during the first few days of drying.

Description of seed.	Grammes of H_2O per 100 grm. of seed.	Cubic centimetres of CO_2 per 100 grm. of seed.	Ratio CO_2/H_2O in tissues of seed.
Peas fresh from the pod	50	54	108/100
Peas after 4 days in laboratory air	22	145	660/100
Beans fresh from pod	60	51	85/100
Beans after 1 day's drying in laboratory air	56	46	82/100
Beans after 4 days' drying in laboratory air	51	41	80/100

In comparison with the above results the following Table gives those obtained in a second series of experiments made to determine the CO_2

content of similar seed during ordinary germination on damp sand after complete air drying in the laboratory:—

Table IX.—The CO_2 Content of Beans (*Vicia faba*) and Peas (*Pisum sativum*) while germinating.

Description of seed.	Grammes of H_2O per 100 grm. of seed.	Cubic centimetres of CO_2 per 100 grm. of seed.	Ratio CO_2/H_2O in tissues of seed.	Growth.
Peas after 18 hrs. germinating	67	64	96/100	None
,, ,, 25 ,,	67	41	61/100	None
,, ,, 39 ,,	70	43	62/100	Sprouting
,, ,, 64 ,,	70	39	55/100	,,
,, ,, 97 ,,	65	16	24/100	,,
Beans after 24 hrs. germinating	58	20	34·5/100	,,
Beans after 7 days in germinating conditions	—	—	41/100	,,
Beans after 5 days without testas in germinating conditions ...	—	—	16·5/100	,,

The experiments lead to the conclusion that in the maturing seed, in the case of beans and peas, the CO_2 content of the tissues is higher than that under which actually germination takes place. In short, so far as these experiments have gone, it would seem that where the CO_2 content of the tissues is above a certain point germination does not occur and that the CO_2 content must fall below this point before germination takes place.

Section IV.—*Contrast of Depressant Action of High Partial Pressures of Carbon Dioxide with Stimulatory Effect of Low Partial Pressures. Carbon Dioxide considered as a Narcotic Agent.*

From the experiments already described it is definitely shown that the phenomenon of non-germination induced in the seed by CO_2 is one of temporary inhibition resulting in a condition strikingly similar to that of narcosis. The interesting question therefore presents itself as to how far this depressant action of carbon dioxide can be regarded as true narcosis.

Looking back, in the first place, through the history of previous work, it has to be noticed that the following results have been recorded as to the effect of carbon dioxide on the growth activity of plants:—

De Saussure (3) in 1804 found that an atmosphere containing 8 per cent. CO_2 restrained the growth of peas. Montemartini (4) found that over 7 per cent. CO_2 depressed the growth activity in the roots of peas. Chapin (5) in 1902 confirmed this. Böhm (6), Dr. Drabble (7), Prof. Farmer (1), and

Brown and Escombe (2) have also conducted experiments tending to show the restraining effect of carbon dioxide on growth. Dr. Drabble and Miss Lake in 1905 demonstrated the stimulation effect of small partial pressures of CO_2, observing that the growth in the length of pea roots was more rapid in 4 per cent. CO_2 than in air and than in percentages greater than 7 per cent. of CO_2.

Here it will be observed that there are two classes of effects recorded; an effect of retardation by higher percentages, and an effect of stimulation by lower percentages of CO_2. The stimulatory effect of small doses is a general property of narcotic agents. A further series of experiments was therefore arranged to test the effect of CO_2 in various proportions below the inhibitory percentage on the germination and growth of *Brassica alba* and *Hordeum vulgare*. The results* obtained with *Brassica alba* are shown in the following Table.

Hordeum vulgare gave similar results.

Table X.—Results obtained in Growth of 10 White Mustard (*Brassica alba*) Seeds under increased Partial Pressures of CO_2, showing the Stimulatory Effects of Low Percentages, rising to a Maximum and then declining towards Inhibition.

Percentage of CO_2 in the atmosphere in each case.	Increase in weight expressed in percentages of original weight of seed.	Average length of growth at termination of experiment in centimetres.
0	23·3	3·8
2	16·0	4·0
3	34·0	4·4
4	23·0	4·3
5	9·0	3·5
10	8·0	2·0
(25 per cent. CO_2 gives complete inhibition)		

This experiment was conducted in a dark room. Average temperature, 16·5° C.

In the foregoing Table it will be observed that the first effect of carbon dioxide is one of stimulation in low percentages. This increases to a maximum which, at the temperature used, is obtained at about 3 per cent. or slightly over, and then declines again through a restraining effect to complete inhibition.

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* The rate of germination was not increased by the low percentages of CO_2 in this experiment, but as has been shown in the case of beans and peas in Table IX, the actual CO_2 content of the seeds is high and falls from an initially inhibitory value as germination proceeds. We should not expect, therefore, small doses of CO_2 in the atmosphere to have a marked stimulatory effect, if any, upon the rate of germination, though their effect upon growth after the escape of the initial high partial pressures of CO_2 in the seeds is clear.

We appear, therefore, to have in view results confirmatory of the hypothesis that we are here dealing with the effect on germination and growth of a true narcotic agent, and that the results induced by CO_2 in the resting seed are a phase of narcosis.

Section V.—*Influence of* CO_2 *in Enforcing Dormancy in Certain Seeds which do not Naturally have a Long Dormant Phase. Seeds of* Hevea brasiliensis.

A considerable amount of work has been done in the past—work which is well summarised by Becquerel (8)—upon various effects produced in dry seeds by sealing them in various gases and vapours, including CO_2. Becquerel discounts the value of part of this work on the ground that it has been conducted on seeds with impermeable testas, so that the gases used could not be considered to have reached the plant embryo.

In a number of experiments conducted during this inquiry on seeds with naturally permeable testas, and on rapidly deteriorating seeds in which the testas may be assumed to be at least partially permeable, carbon dioxide was found in nearly all cases to have certain definite effects, such as might have been expected from the foregoing experiments conducted upon wet seeds in germinating conditions.

The results of this work, which is still in progress, have not yet been correlated, but one aspect of them may be referred to here, as bearing directly upon the central problem discussed in this paper.

One of the most rapidly deteriorating seeds is that of *Hevea brasiliensis*. In planting in the tropics it is found that it is always desirable to put the seed in the ground within a fortnight, and Mr. C. Curtis, late director of the Botanical Gardens, Penang, from whom the seeds used were obtained, writes that even in such circumstances 70 per cent. germination is considered good. This rapid deterioration of the seed has been a difficulty in the recent extension of rubber plantations, and the question of the best conditions for preservation in packing and export has been an important one, leading to practical research. The seeds are at present usually packed in ground charcoal and ashes. Their size is about that of an average acorn or larger. They have easily permeable testas and a high water content, and while living they were found to be respiring very rapidly. They were also found to be very intolerant of drying. The seeds in the experiments considered in this research were enclosed in hermetically sealed flasks under various conditions, and it was found as the outcome of a number of experiments that when they were sealed in the proportion of 40 to 50 seeds to 1200 c.c. of air the following results were obtained:—(1) A partial pressure of CO_2 of 40–45 per cent. was created in the flasks by the life processes of the seeds, and (2) there was a marked prolongation in their period of vitality.

In the following Table the results of two experiments are given. The imported seeds, when received in this country, were necessarily some weeks old. The temperature at which germination tests were conducted was 27° C. in a thermostat:—

Table XI.—Showing prolonged Dormancy of *Hevea brasiliensis* Seeds sealed in flasks as described. Flasks opened after 50 days. A test germination, begun at the time of receipt of the seeds, gave 40 per cent. germinations.

How kept during 50 days.	Analysis of atmosphere on opening flasks after 50 days.			Percentage of germinations after 60 days.
	CO_2.	O_2.	N_2.	
	per cent.	per cent.	per cent.	per cent.
Experiment 1—				
50 seeds in air in 1200 c.c. sealed flasks	45	1·3	53	40 (good plants)
50 seeds in air in 1200 c.c. open flask	—	—	—	8
50 seeds in air in commercial packing as sent from tropics	—	—	—	16
Experiment 2—				
20 seeds in air sealed in 500 c.c. flask	40	4·0	56	40 (good plants)
20 seeds in nitrogen sealed in 500 c.c. flask	41	1·0	58	25 ,,
20 seeds in air in 500 c.c. open flask	—	—	—	0

The first of the above experiments took place over the months of November and December. The flasks were kept in a temperature varying from 10° to 15° C.

The second experiment took place during December and January. The flasks were kept in the laboratory, the temperature varying from 18° to 13° C. There was considerable internal pressure when the flasks were opened in both experiments.

In a third experiment the period during which the seeds were kept from date of importation was prolonged to 90 days. The average temperature was considerably higher, the months over which the experiment extended being September, October, and November. In this case 10 per cent.

germinations were obtained with seeds sealed in air in the proportion mentioned against *nil* with seeds kept in commercial packing, *nil* with seeds kept in open air, and *nil* with seeds sealed in nitrogen.*

In the foregoing experiments it will be observed that large seeds enclosed in permeable seed coats and sealed with a definite proportion of air in an impenetrable outer envelope were being dealt with. In these conditions, where the life processes of the seeds resulted in the creation in the flask of a partial pressure of CO_2 of 40–45 per cent. the vitality of the seeds was markedly prolonged.

A conclusion which Becquerel reaches, as the result of his researches, is that in all cases of longevity in dry seeds the testas are exceptionally strong and impermeable. The problem of the dry seed enclosed in an impermeable or almost impermeable testa has certain striking affinities—in that gaseous exchange in either direction is hindered or prevented—to that of the wet seed, though in apparently good germinating conditions, which does not germinate. But with the former problem we are not at present directly concerned in this research.

Section VI.—*Biological Importance of Dormancy in Moist Seeds.*

The seed is a comparatively late arrival in geological time, and the perfecting of its function has of necessity been a great point in the struggle for existence amongst plants. A leading cause in the success of the Angiosperms, as Prof. Seward has pointed out, has consisted in the efficiency of the arrangements for nursing the embryo. There can be no doubt that a ruling factor in this efficiency has been the adjustment of all the life processes of the moist resting seed to the end of attaining a fit time for germination. It is suggested by these experiments that the presence of carbon dioxide in the tissues of the embryo acting as a restraining and inhibiting agent on the life processes of the seed, and as a dominant factor in relation to the oxygen stimulus, has been utilised in attaining this efficiency of the latent seed for which fit conditions of germination have not yet arrived. The various structures of the testa and its behaviour under different conditions in regulating the gaseous exchanges appear to be important factors in obtaining the necessary adjustments to natural conditions.

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* The favourable results obtained in these experiments in prolonging the vitality of these rapidly deteriorating seeds were greatly in excess of those which are secured by present commercial methods of packing for transport and import. In experiments on a large scale the seeds might be simply sealed (in the proportions of air mentioned) in large carboys, such as are used for the transport of distilled water, covered with wicker or wire netting. In case of too high an internal pressure, arising from overfilling with seeds, a simple form of safety valve might be inserted in the sealing.

Emphasis may properly be laid on the fact that it is these adjustments of the moist seed when in apparently suitable conditions of temperature, moisture, and oxygen supply, while awaiting the fit time for germination, and not so much the adjustments of the resting dry seed, that have formed the central problem of seed life in conditions of nature. The maintenance of latency when the moist seed is in conditions of medium temperature, oxygen supply, and moisture, has been the problem of the maturing seed on the parent plant. It has been the problem of a large proportion of native seeds which fall upon the ground in summer and autumn, but whose fit time for germination does not arrive till the following spring. It has, beyond doubt, been the problem also of many species of plants in the struggle for existence whose chances therein must have often been increased manyfold by the capacity of their seeds to lie dormant in the ground for indefinite periods, ready to resume activity with sporadic germination when suitable conditions arise such as, for instance, occurred in the case of the *Brassica alba* seeds of these experiments when the testas became dry or ruptured.

Section VII.—*Summary and Conclusions.*

Part I.—Experiments were conducted showing that the germination of seeds is retarded or inhibited by high partial pressures of CO_2 in the atmosphere. This retardation and inhibition produced by CO_2 was shown to be unaccompanied by injury. The seeds used in these experiments fall into two classes. In the first class the seeds germinated at once after removal from the inhibitory CO_2 pressures (beans, cabbage, barley, peas, onions). In the second class the inhibition continued indefinitely after the removal of the inhibitory CO_2 pressures, and is terminated only by complete drying (and rewetting), or by the removal of the testa. In this class a lowering of the permeability of the testa to gases under the influence of CO_2 is indicated, a change which would have two results: (1) a reduction in the amount of oxygen reaching the embryo; and (2) a relative rise in the actual CO_2 pressure in the embryo tissues. The condition of prolonged inhibition after removal to air produced in *Brassica alba* is strikingly suggestive of the condition of seeds often met with in nature, the germination of which is delayed in spite of suitable conditions of temperature and water. The results obtained in the laboratory with *Brassica alba* seeds were reproduced in the soil in natural conditions by CO_2 arising from decaying vegetable matter. The high CO_2 content of the soil air in these experiments was found to continue for a considerable period. Attention was called to the importance of these facts in agriculture.

Part II.—A long series of experiments was carried out to determine the relation of carbon dioxide inhibition in seeds to temperature and to oxygen supply. Low temperatures and low oxygen supply were both found to increase the inhibitory value of given partial pressures of CO_2, while inversely the inhibitory value of given carbon dioxide pressures diminishes with a rise of temperature and with a rise of oxygen pressure. The probable relation of these facts to the dormancy of the moist seed in natural conditions was pointed out.

The arrested development of maturing seeds on the plant was shown not to be due to lack of moisture or to any physiological insufficiency. The seeds in this stage were shown to contain in their tissues more CO_2 than seeds normally germinating contain at the moment of sprouting. The presence of the testa was shown constantly to retard the germination both in seeds taken from the parent plant before natural drying and in seeds after complete drying and storing. Attention was drawn to the correlation found to exist between the viviparous habit and the absence of seed coats.

Carbon dioxide has been considered as a narcotic agent. Previous work on the action of CO_2 upon growth has been quoted. The stimulatory effect of low partial pressures, rising to a maximum with increasing pressures and then declining to inhibition with higher pressures of CO_2, has been demonstrated by experiments with *Brassica alba* and *Hordeum vulgare* germinated in the dark.

In the case of certain rapidly deteriorating seeds (*Hevea brasiliensis*) the carbon dioxide naturally produced by respiration of the seeds in a closed flask rose to 40 per cent. and the presence of this was found to be accompanied by a marked prolongation of vitality in the seeds. This prolonged vitality was far in excess of that reached with the present commercial method of packing these short-lived seeds for export.

When we correlate the results of these different lines of experiment we seem to get in various directions evidence of the importance of carbon dioxide pressure as a controlling influence in the biology of seeds. This influence may be formulated briefly in the following principles:—

(1) The resting stage of the moist seed is primarily a phase of narcosis induced by the action of carbon dioxide.

(2) Both the arrested development in the case of the moist maturing seed on the plant, and the widely occurring phenomenon of delayed germination in the case of the moist resting seed, which does not germinate although in apparently suitable conditions of temperature, moisture, and oxygen supply,

are related to an inhibitory partial pressure of carbon dioxide in the tissues of the embryo.

(3) Germination when it takes place is related to a lowering of the value of this inhibitory partial pressure of carbon dioxide in the tissues.

(4) The inhibitory value of a given carbon dioxide pressure diminishes with a rise of temperature.

(5) The inhibitory value of a given carbon dioxide pressure diminishes with a rise of oxygen pressure.

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JOHN LUBBOCK, BARON AVEBURY—1834–1913.

THE first Lord Avebury, for many years better known as Sir John Lubbock, died on May 28 last, in his 80th year. He was the eldest son of the third Baronet and Harriet, daughter of Captain Hotham, of York. He was educated at Eton, but left at an early age to join his father in the family bank. He married firstly Ellen the eldest child of Peter Hordern, and some years after her death, in 1879, Alice Augusta Laurentia, daughter of the late General A. A. Lane-Fox Pitt-Rivers, and grand-daughter of the second Baron Stanley of Alderley. In 1865, he succeeded his father as fourth baronet, five years later he became Member of Parliament for Maidstone, and held this seat until 1880, when he was elected representative of the University of London. This seat he held until 1900, the date when he was removed to "another place," as Baron Avebury.

Lord Avebury took an active but restricted part in politics. His most prominent efforts were directed to the establishment of Bank Holidays, but he devoted much time and attention to educational questions and social reform. Without having had a University training he was yet peculiarly fitted to be a representative of a University, being a man of wide culture as well as a very competent man of business. For many years he was head of the great banking company, Robarts, Lubbock and Co., and by his tireless activity and ceaseless care for detail, he became a very prominent man in City circles. This attention to detail and his knowledge of procedure made him an admirable President; and, indeed, he seems to have presided over nearly every scientific society and countless mercantile associations. At various dates he was President of the British Association (Jubilee Year), the Entomological Society, the Ethnological Society, the Linnean Society, the Anthropological Institute, the Ray Society, the Statistical Society, the African Society, the Society of Antiquaries, and the Royal Microscopical Society. He was also the first President of the International Institute of Sociology, the President of the International Association of Prehistoric Archæology, the International Association of Zoology, the International Library Association, the London University Extension Society, and the first President of the Institute of Bankers, President of the London Chamber of Commerce, and of the Central Association of Bankers.

For eight years he was Vice-Chancellor of the University of London, and he was also Principal of the Working Men's College. He sat on many a Royal Commission, and left his mark on those on the Advancement of Science, on Public Schools, on International Coinage, on Gold and Silver, and on Education. He was perhaps less happy as President of the Committee which selected the designs for our present coinage.

At the time of his death, Lord Avebury, although he retained a house in London, had given up his house in St. James's Square, and he died at Kingsgate Castle, Kent. Another of his country residences was High Elms, Down, and it may have been the association of Darwin and Avebury at this small Kentish village that first attracted Lord Avebury's attention to natural history.

One of his first books, and perhaps one of the most stimulating, was 'The Origin of Civilisation and the Primitive Condition of Man,' now in the sixth edition, a book which aroused interest and research in the past in many quarters. It was characteristic of him when he had to select a title for his peerage to choose that of Avebury, the preservation of whose prehistoric remains he had taken so large a part in securing. At the time of his last illness he was engaged in revising and partly rewriting a seventh edition of his well-known 'Prehistoric Times.'

Without being a great researcher, Lord Avebury took a very prominent part in encouraging the research of others. Of his more scientific works, perhaps his monograph (published by the Ray Society) 'On the Collembola and Thysanura' has proved most useful; for a long time it was the authoritative work on these lowly insects, and still is so, especially with regard to the Collembola, whose distinction from the Thysanura was first recognised by the author. But many of his other works passed into numerous editions: 'British Wild Flowers, considered in Relation to Insects,' reached the sale of 11,000 copies; 'Ants, Bees, and Wasps' passed into the seventeenth edition; and his works on 'Seedlings' and on 'Buds and Stipules' contained much that is valuable and well worthy of record.

He wrote two geological works which are still used with profit by students of the Universities; one on 'The Scenery of Switzerland,' and the other, published ten years ago, on 'The Scenery of England,' and several treatises on more strictly economic lines. His works on Coins and Currency, on Free Trade, and on Municipal, and on National Trade, occur to one's mind. But apart from these more or less technical publications, Lord Avebury had a genuine "flair" for writing books which the public want. Both parts of 'The Pleasures of Life' sold over 200,000 copies, and Part I over a quarter of a million, besides being issued in no less than forty foreign editions. 'The Use of Life' and 'The Beauties of Nature' were hardly less successful, and everyone will remember his "Hundred Best Books."

As the foregoing will show, Lord Avebury was a man of singularly diversified activities and extreme width of interest. That he should find occasion in the middle of a busy business career to do the work he did is indeed amazing, but he was precise and very business-like, and knew how to make the most of his time.

He had after his name an alphabet of Honorary Degrees and memberships of Learned Societies. It need hardly be said that he was covered with honours too numerous to enumerate. He was Lord Rector of the University of St. Andrews, Trustee of the British Museum, and Foreign Secretary to the Royal Academy. He served five distinct periods on the Council of the Royal Society, the last being in the year 1906–7, and was three times Vice-President. He was Commander of the Legion of Honour, and held the German "Ordre pour le Mérite."

A. E. S.

PHILIP LUTLEY SCLATER—1829–1913.

Philip Lutley Sclater was born in November, 1829, at Tangier Park, in Hampshire, where his father, Mr. William Lutley Sclater, then resided, though he shortly after moved to Hoddington House, another estate in the same county, not far from the old home of Gilbert White, where his boyhood was passed.

In 1842 he went to Winchester College and was elected a scholar of Corpus Christi College, Oxford, in 1845, but being under age was not called into residence at the University until the following year. At Oxford he devoted his studies chiefly to mathematics, but at the same time he occupied much of his spare time in the pursuit of natural history, his speciality, as in after life, being ornithology. While at Oxford he was fortunate in becoming acquainted with H. E. Strickland, and at his house he met John Gould, shortly after the return of the latter from Australia. It was from them that he received his first serious instruction in ornithology, and it was during his Oxford days that he commenced his collection of birds.

In 1849 he took his degree, obtaining a first class in Mathematics and a pass in Classics, but he remained for two years longer at college before proceeding to his M.A. degree. During this time he also studied modern languages and became familiar with French, German, and Italian, spending as much of his time as he could spare on the Continent. At Paris he made the acquaintance of Prince Charles Bonaparte, at whose house he was a constant visitor, and thus he received a further stimulus in his favourite pursuit of ornithology.

In 1855 Sclater became a Fellow of Corpus Christi College, Oxford, and was called to the Bar by the Honourable Society of Lincoln's Inn and went on the Western Circuit for several years. In 1856 he visited America, in company with a friend, and attended the American Association for the Advancement of Science, at Saratoga, after which they proceeded to Niagara and the Great Lakes, and on foot to the upper waters of the St. Croix River, thence descending in a birch-bark canoe to the Mississippi. They finally returned to Philadelphia, where Sclater spent some time studying the fine collections at the Academy of Natural Sciences and meeting John Cassin, Joseph Leidy, John le Conte, and other well-known naturalists, returning to England about the end of the year.

He now took up his residence in London, continuing his studies in natural history and also practising at the Bar. He was a constant attendant at the meetings of the Zoological Society of London, of which he had been previously elected a Fellow, and in 1857 became a Member of the Council. In 1859 Sclater, in company with his friend E. C. Taylor, made an expedition to Tunis, visiting the breeding places of the vultures, eagles, and other Raptores and making considerable collections.

About this time Mr. D. W. Mitchell, who had been Secretary to the Zoological Society, was appointed to superintend the new Jardin d'Acclimatation in Paris; thus the post became vacant, and Owen and Yarrell, influential members of the Council, induced Sclater to apply for it, and at the Anniversary Meeting in 1859 he was unanimously elected. On his appointment he found that a considerable re-organisation of the Society's affairs was necessary, the 'Proceedings' and 'Transactions' were sadly in arrear, and the gardens themselves were much neglected. He at once set to work to reform these matters, and as a result the prosperity of the Society vastly increased. The number of Fellows was augmented from about 1700 in 1859 to above 3000 when he resigned his post in 1902, and, similarly, the income rose in the same period from £14,000 to £30,000 and both the buildings in the Gardens and the offices in Hanover Square were replaced by much more suitable and commodious structures, the library also received great attention and now became an important feature of the Society. From 1874 to 1876 he became private secretary to his brother (then the Right Honourable Sclater-Booth, M.P., and afterwards Lord Basing), when he was President of the Local Government Board in Mr. Disraeli's Administration.

The British Ornithologists' Union was established in 1858 for the study of general ornithology and Sclater was invited to become Editor to the first series of its quarterly journal, 'The Ibis.' Volume I appeared in 1859, and the first series was completed in 1865. The next six volumes were edited by Prof. A. Newton, and the third series by Osbert Salvin. From 1877 Sclater again became Editor, either alone or in company with a partner, till the end of the ninth series in 1912, and during this time he contributed many valuable papers to the Journal. In 1908, on the occasion of the Jubilee, Sclater, together with the three other surviving founders, F. D. Godman (President), W. H. Hudleston, and P. S. Godman, received the gold medal of the Society.

With the British Association for the Advancement of Science he had a long connection, and attended many of the meetings after he became a member in 1847, including the visit to Montreal in 1884 and South Africa in 1905. For several years he was Secretary of Section D, and at the Bristol meeting in 1875 was its President, and delivered an address on "The State of our Knowledge of Zoological Geography," a subject which had hitherto been much neglected. In geography he took a special interest; he became a life member of the Geographical Society, and was a constant attendant at its meetings. He resigned the Secretaryship of the Zoological Society in 1902 after forty-three years' tenure of that office, and retired to his country house, Odiham Priory, in Hampshire, but was still a frequent visitor at both the Natural History Museum and the Library of the Zoological Society till shortly before his death. He continued a constant attendant at the dinners of the British Ornithologists' Club, at which he usually presided. At the last meeting, held on June 11, 1913, he was presented by the club with an address, signed by nearly all the members, and a piece of plate, in recognition of his services during the past twenty-one years, but he was, unfortunately, too unwell to be present, as he was suffering from a carriage accident, from the effects of which he died on June 27.

Sclater married in 1862 Jane Anne Eliza, youngest daughter of Sir David Hunter-Blair, Bart., of Blairquhan, Ayrshire, and leaves a widow and three sons and one daughter.

With a view to obtain collections of natural history, Sclater assisted in promoting researches in foreign parts. Amongst these may specially be mentioned Sir H. H. Johnston's expedition to Kilimanjaro, Prof. Balfour's visit to Socotra, and many others. Sclater likewise travelled in many parts of Europe and North America, visiting the museums, and making the acquaintance of the principal zoologists.

As before mentioned, he commenced his collection of birds while an undergraduate at Oxford, at that time intending to include those from all parts of the world, but afterwards resolved to confine himself to Central and South America alone, limiting himself to the orders Passeres, Picariæ, and Psittaci. This collection, containing 8824 specimens, representing 3158 species, including many types, was ultimately acquired by the Natural History Museum.

Sclater received the honorary degree of Doctor of Philosophy from the University of Bonn in 1860, and was made a Doctor of Science by the University of Oxford in 1901. He was elected a Fellow of the Royal Society in 1861, and served twice on the Council, was likewise a Fellow of the Linnean, Geographical, and Geological Societies, and a member of several other scientific societies both at home and abroad.

Amongst the works published by Sclater may specially be mentioned 'A Monograph on the Tanagrine Genus *Calliste*,' 'Zoological Sketches,' by J. Wolf, with notes by P. L. Sclater, 'Exotic Ornithology,' by P. L. Sclater and Osbert Salvin, and the 'Book of Antelopes,' by P. L. Sclater and Oldfield Thomas. In addition to these, he published over 1200 papers in various periodicals, chiefly on birds and mammals, besides many others in conjunction with Osbert Salvin, Forbes, and O. Thomas, etc. His last paper in the 'Ibis' was issued in the January number, 1913, while his first in the 'Zoologist' in 1844.

F. D. G.

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Arranged for a Committee of the Royal Society under the Superintendence of HERBERT McLEOD, LL.D., F.R.S., Director of the Catalogue.

WHEN the plan for a Catalogue of Scientific Papers was drawn up in 1857 by the Royal Society, it was also contemplated that a Subject Index to the papers should be made. During the compilation of the well known twelve volumes of the Catalogue of Authors (1800—1883) much material was collected for the Subject Index by the Committee of the Society which had charge of the work, but nothing was printed.

In 1898 it was determined to undertake the continuation for the period 1884—1900, and at the same time to prepare material for a Subject Index for these last seventeen years of the century. It was afterwards decided that one Index should be made to the papers of the whole of the Nineteenth Century; and to bring this Index into relation with the International Catalogue of Scientific Literature, which deals with publications subsequent to 1900, the Index was to be arranged in accordance with the schedules of the different sciences which form the basis of the International Catalogue.

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The Subject Index was arranged to be published as separate Index-volumes for each of the seventeen Sciences of the Schedules of the International Catalogue, viz. Mathematics, Mechanics, Physics, Chemistry, Astronomy, Meteorology, Mineralogy, Geology, Geography, Palaeontology, Biology, Botany, Zoology, Anatomy, Anthropology, Physiology, and Bacteriology; but for the present only the volumes relating to the first three of these sciences have been printed.

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The first part of the volume devoted to Physics has now been published; it deals with Generalities, Heat, Light, and Sound, and contains 33,344 entries from 1,261 serials. The second part of the Physics volume, devoted to Electricity and Magnetism, is now ready; it contains 23,300 entries.

Each volume contains a list of the serials from which the entries are taken, and indications are given of the principal British libraries in which they may be consulted.

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